Neuroplasticity and -Modulation in Tinnitus: Understanding Brain Imprints and Suppression of a Phantom Sound

Thesis (cumulative thesis)

presented to the Faculty of Arts and Social Sciences

of the University of Zurich

for the degree of Doctor of Philosophy

by Patrick Neff

Accepted in the fall semester 2017

on the recommendation of the doctoral committee:

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Zurich, 2017

Abstract

In the thesis at hand studying tinnitus, both inconsistent results of cortical morphology related to tinnitus and a novel approach of acoustic stimulation for tinnitus relief are treated.

After giving a general introduction as well as contemplations about prevalence and relevance of this phantom sound percept, concurrent models and the state of research are introduced briefly.

Aiming at study 1 reported in the empirical part of this thesis, an analysis of a large sample of tinnitus patients by means of an innovative, observer-independent approach of neuroanatomy, recent and concurrent studies of tinnitus-related neuroanatomy are introduced and discussed comprehensively.

Subsequently, the relevant literature concerning acoustic stimulation in tinnitus is reviewed and discussed towards the inception of a novel stimuli class, namely amplitude modulated sounds, for short-term tinnitus suppression and potential use in long-term therapeutic sound therapies for tinnitus relief. These considerations led to the inception of a first exploratory study (study 2) which tested feasibility and safety of the approach.

Building on insights derived from study 2, study 3 further tested the approach's efficacy in tinnitus suppression while also comparing subjective reactive ratings of the sounds.

Finally, results are discussed in a broader context of ongoing research and general conclusions as well as outlooks to future research endeavors are provided.

Zusammenfassung

In dieser Dissertationsschrift werden widersprüchliche Befunde kortikaler Morphologie von Tinnitus abgehandelt sowie eine neuartige Methode zur akustischen Stimulation vorgestellt.

Nach einer allgemeinen Einführung in das Phantomgeräusch wird der aktuelle Forschungsstand diskutiert.

Im Hinblick auf die erste empirische Studie (Studie 1) werden frühere und aktuelle Studien zu tinnitusspezifischer Neuroanatomie vorgestellt und ausführlich diskutiert.

Im Weiteren wird die Literatur zur akustischen Stimulation bei Tinnitus eingeführt und diskutiert. Die Diskussion wird dann übergeleitet zum Einsatz einer neuen Stimulusklasse in Form von amplitudenmodulierten Klängen zur kurzzeitigen Unterdrückung des Tinnitus. Die hier dargelegten Überlegungen führten zum Design einer ersten explorativen Studie (Studie 2), welche primär die grundsätzliche Brauchbarkeit und Sicherheit der neuen Stimulusklasse prüft.

Darauf aufbauend, testete Studie 3 die spezifische Effektivität des Ansatzes im Hinblick auf Tinnitusunterdrückung und verglich zusätzlich subjektive Bewertungen der Klänge.

Abschliessend werden die Resultate der Studien in einem breiteren Kontext aktueller Forschung diskutiert, allgemeine Schlussfolgerungen gezogen sowie ein Ausblick zu möglichen zukünftigen Forschungssträngen dargelegt.

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Acknowledgments

I would like to dedicate this doctoral thesis to Martin Meyer for supporting me on all levels throughout my doctorate and the inception of this thesis: Without his trust, dedicated mentorship, scientific and financial support my first steps in academia would not have been as insightful and productive. Thank you, Martin, for helping me on this path.
Furthermore, I would like to thank Tobias Kleinjung and Steffi Weidt, the other members of the multidisciplinary tinnitus research group in Zurich, for their trust and support all these years.
I am also deeply grateful to Winny Schlee, not only for collaborating on the acoustic stimulation project and being on my committee, but also for integrating me in the European tinnitus research network.
In Regensburg, I am thankful to Martin Schecklmann, Berthold Langguth, Susanne Staudinger and the rest of the team for their help and excellent hosting.
Finally, I would like to thank Moritz Daum for complementing my committee.
All of this work would not have been possible without the help of all the volunteers suffering from a partly tantalizing condition: I can only admire their compliance and endurance during partly tedious procedures like MRI scanning or acoustic stimulation in and around their tinnitus frequency.
I thank the URPP "Dynamics of Healthy Aging" for sponsoring my position and the great institutional support: Special thanks go to Christina Röcke, Sandra Jaster and Tobias Hoffmann.
Further, I would like to thank all my colleagues especially Nathalie Giroud, Dominik Güntensperger, Matthias Keller, Ira Kurthen and Laura Jagoda.
Outside the core lab, I would like to thank Franz Liem and the team of the Institute for Computermusic and Sound Technology at the Zurich University of Arts, especially Jan Schacher, for our valuable collaborations.
I am also thankful to my master students and interns for their extraordinary dedication and continued inspiring relationships.
Most of all, I would like to thank Simone - without her patience, emotional and physical support throughout the difficult times following my heavy accident, I would not have been able to write this thesis: I am deeply, deeply indebted to you.

Glossary

AM : amplitude modulation

CC : corpus callosum

CSA : cortical surface area

CT : cortical thickness

CV : cortical volume

dmPFC : dorsomedial prefrontal cortex

DTI : diffusion tensor imaging

EEG : electronencephalography

FCDS : forced-choice double-staircase

FM : frequency modulation

GM : gray matter

HG : Heschl gyrus

HL : hearing level

MEEG : magneto-/electroencephalography

MEG : magnetoencephalography

MML : minimum masking level

MOA : method of adjustment

MRI : magnetic resonance imaging

NAc : nucleus accumbens

NBN : narrowband noise

PAC : primary auditory cortex

PLR : pitch likeliness method

PT : pure tone

RI : residual inhibition

ROI : region of interest

SBM : surface-based morphometry

SL : sensation level

SPL : sound pressure level

SPM : statistical parametric mapping

TCD : thalamo-cortical dysrhythmia

TI : tinnitus sufferer

TQ : Tinnitus Questionnaire

VBM : voxel-based morphometry

vmPFC : ventromedial prefrontal cortex

1 Introduction

In the thesis at hand, first an introduction to tinnitus and its models is given. Second background, methods and open research questions relevant to the empirical work of this thesis, namely neuroanatomy and auditory stimulation in tinnitus, are introduced. Third, the empirical manuscripts are presented, starting with the neuroanatomical study of a large sample of tinnitus sufferers (TI), followed by the two studies on a novel acoustic paradigm for tinnitus suppression possibly mediated via the normalization of aberrant central auditory neurophysiology. Fourth, the results are discussed in the broader context of concurrent tinnitus research. Finally, further ongoing research is introduced and future possible fruitful avenues of tinnitus research are envisioned.

1.1 Tinnitus

1.1.1 Historical Frame

The phenomenon of a ringing, whistling, sizzling, humming, hissing, buzzing, whooshing, roaring, fizzing, crackling, cricketing, knocking, or pulsing sound in the absence of an external source (Eggermont and Roberts, 2004) is known and reported since archaic or antique times (Steiner, 2012; Dietrich, 2004). Yet, there is still debate about its first mentioning: Until recently it has been agreed upon that the earliest reference is attributed to the Papyrus Ebers (a translation of ancient Egyptian scrolls) studied by the German Egyptologist Georg Ebers (Dietrich, 2004). According to this arguable first mentioning on ancient Egyptian scrolls stemming from the 17$^{\text{th}}$ dynasty (1650-1532 B.C.), possibly even based on older sources, tinnitus is described as a 'bewitched ear'. However, there is an ongoing rampant discussion about an exact translation and appropriateness to context of the respective passage. Therefore Dietrich (2004) continues to challenge the ancient Egyptian source and proposes that the earliest solid mentioning can rather be seen in the Corpus Hippocraticum authored by Hippocrates the Greek physician living from 460 to 377 B.C.. In this manuscript tinnitus is not only described as '$\eta\chi o\sigma$' (echos, meaning

sound), 'βo$\mu\beta$oσ' (bombos, meaning 'buzzing') and 'ψoϕoσ' (psophos, meaning 'slight sound') but also linked to hearing loss, which is astonishing given established concurrent models of tinnitus where hearing loss is a prerequisite leading to altered physiology throughout the auditory pathway and the brain (Eggermont and Roberts, 2004). Adding to this fundamental discussion about etiology and pathogenesis of tinnitus, here in the light of the historical considerations, the Talmud is also worth mentioning , where Titus allegedly was cursed with tinnitus after the destruction of the temple (Dan, 2005). In these passages tinnitus was not only described as a buzzing sound similar to a 'gnat' and temporarily maskable with a jackhammer sound, but it has also been attributed to a disease of the brain, which is again astonishingly modern in its reasoning while certainly the central role of this organ in general human biology was not in full grasp yet. Finally, Pliny the Elder coined the term tinnitus derived from the Latin verb tinnire (to ring) which afterwards remained the common description of the phantom sound in absence of any external physical source until today (Morgenstern, 2005).

Besides the historical aspects of early mentionings, first models of etiology and pathogenesis, early attempts or recommendations towards a cure or symptoms alleviation, and finally the inception of a universally valid terminology, the question whether tinnitus has always been haunting mankind or if it is a phenomenon of modern affluent societies with noisy as well as stressful environments remains a hot potato. With a growing deeper understanding of pathogenesis and -physiology of tinnitus (Elgoyhen et al., 2015; Langguth et al., 2013), and especially first causal relationships between noise exposure with hearing pathologies including tinnitus (e.g., leisure music exposure of teenagers (Sanchez et al., 2016)), it can thus be concluded that we may be on the brink of a veritable tinnitus epidemic. This conclusion is substantiated by an aging Western demographic and related presbycusis, a mostly inevitable form of (peripheral) hearing loss in late life, leading to an increased prevalence of tinnitus (Hoffman and Reed, 2004). Beyond hearing loss, while not being a direct cause in the most cases, stress and its adverse effects on physiology but also mood and cognition does exacerbate the severity of and reaction to the phantom sound (Mazurek et al., 2012). Taken together, evidence is accumulating that, while tinnitus has been recognized in the past throughout documented history, modern environments and lifestyles account for, instead of only giving the impression of, a higher prevalence of tinnitus in our modern times. It has been also established that tinnitus is somehow

always caused by hearing loss or other pathologies of the auditory system (Eggermont and Roberts, 2004) and that alternative (metaphysical) theories considering tinnitus as a symptom of a deeper-rooted existential unease in an alienating sphere or mode of life (Steiner, 2012) can be dismissed.

1.1.2 Phenomenology, Prevalence and Relevance

As seen in the last paragraph, tinnitus is predominantly defined as "the perception of sound(s) in the absence of an external (physical) sound source" Eggermont and Roberts (2004); Erlandsson and Dauman (2013). Alternatively, tinnitus can be regarded as the "absence of silence in silence" as Aage R. Møller elegantly circumscribes the phenomenon (personal communication). Two separate forms of the phantom sound have to be distinguished: the rare form of 'objective' tinnitus is characterized by an organically caused sound in the ear, which is also audible to an external listener (e.g., an audiologist, (Langguth et al., 2013)), whereas the typical form, 'subjective' tinnitus, is only audible by the sufferer and thus fitting the above-mentioned established definition. In addition, the phantom sound perception has to be identified as chronic (i.e., constant presence for at least 12 months after acute and sub-acute phases (Mazurek et al., 2010)) so that the final diagnosis of 'chronic subjective tinnitus', the most common and tantalizing form of the phantom percept with no cure in sight, is applicable. In the remainder of this manuscript (and generally in most literature on the topic of tinnitus) chronic subjective tinnitus is merely termed 'tinnitus', given the fact that it is the most common form by far.

About 35% of the general US population suffers from tinnitus at some point during their lifetime (Jastreboff, 1990). 10–15% report their tinnitus as being frequent or continuous while an estimated 1–2% concurrently suffer immensely under the condition (Langguth et al., 2013). Looking at absolute numbers, approximately 50 million people in the US and 70 million people in the European Union are haunted by tinnitus at this moment (Cederroth et al., 2013). Tinnitus is often accompanied by comorbid symptoms (Langguth, 2011) like insomnia, depression and anxiety, altered cognition (Andersson and McKenna, 2006) and impaired auditory functioning (e.g., speech perception (Moon et al., 2015; Ivansic et al., 2017), auditory attention (Cuny et al., 2004), and sound localization (Hyvärinen et al., 2016)). This further renders the determination of exact prevalence rates, but especially the tinnitus-specific burden of disease, a difficult endeavor. Given the multitude of

causes and manifestations, the wide variety of related and interlocking comorbid symptoms and finally the individual phenotypic suffering profile, tinnitus has to be understood as a highly heterogeneous phenomenon. In recent years, conglomerate research initiatives on a European level (e.g., TINNET, http://www.tinnet.tinnitusresearch.net/) as well as on an international level (e.g., TRI, http://www.tinnitusresearch.org/) emerged to tackle the heterogeneity inherent to tinnitus. Cultivating a spirit of establishing research guidelines, identifying fruitful avenues of research as well as building international research collaborations where specific expertise is concentrated, these initiatives contribute to sane and informed progression of tinnitus research. Partly, this is reflected by respective published guidelines (clinical routine: http:www.tinnitusresearch.org/en/projects/flowchart_en.php), (clinical) study routines (Langguth et al., 2007; Landgrebe et al., 2012), call-to-action papers (Langguth, 2011; Cederroth et al., 2013), and methodological considerations (e.g., (Adjamian, 2014)), and results from the joint database projects (Landgrebe et al., 2010). Despite many efforts and advancements, tinnitus still presents itself as a chronic disease that can not be cured. Anecdotally, this observation may be further substantiated by a change in slogans of the research initiatives: The current European initiative TINNET established in 2014 sports ”An Action for Better Understanding the Heterogeneity of Tinnitus to Improve and Develop New Treatments” whereas the TRI, established more than ten years ago, optimistically states ”Together for a cure!”.

While researchers in various fields are busy day and night, it becomes more and more evident that tinnitus will continue on its rise and that it will become a relevant issue for society especially for the aging demography with related higher occurrence of presbycusis. Additionally, recent studies also hint at risky behaviors in early life possibly facilitating the generation of tinnitus (in later life) (e.g., (Sanchez et al., 2016; Roberts et al., 2010; Eggermont, 2016)). Unfortunately, specific tinnitus prevention, or more accurately put, hearing loss prevention expanded with the addition of tinnitus is completely missing, which is intriguing especially given the irreversibility of chronic subjective tinnitus to date.

1.1.3 Etiology, Pathogenesis and Chronification

Generally, there is augmenting consensus that tinnitus is caused by any form of hearing loss be it objective (Eggermont and Roberts, 2004; Mazurek et al., 2010; Schaette and

Kempter, 2006), or sub-threshold or hidden (Schaette and McAlpine, 2011; Weisz et al., 2006) in most of the cases (>80%, (Baguley et al., 2013)). Following (peripheral) hearing loss, maladaptive plasticity throughout the auditory pathway and the brain is observed ((Elgoyhen et al., 2015), see figure 1.1), culminating in alterations of multiple, parallel, and overlapping brain networks related to various aspects of tinnitus (De Ridder et al., 2014). The generation of the phantom sound, and especially its maintenance in the individual brain, is believed to be highly individual, while the influence of stress (Mazurek et al., 2010), the interplay with depressive symptoms mediated via downregulation of serotonergic circuits disabling thalamic 'noise canceling' in frontostriatal gating models (Rauschecker et al., 2010), or the role of attention (Roberts et al., 2013), may be valid for all tinnitus sufferers as exacerbation factors.

In the following common models of tinnitus etiology, pathogenesis, and chronification are introduced in more detail while, for the sake of brevity and relevance (i.e., to the topics and methods covered in this thesis), not all models are discussed in depth. Looking at these encompassing models, the interested reader is referred to a recent opinion paper which elegantly covers and briefly discusses all current tinnitus models, their interaction, and data which speak in favor of or against the maintenance of these models ((Sedley et al., 2016), see figure 1.2). Notably, all models agree on any kind of hearing loss or, to a lesser extent, peripheral organic disease as the actual origin of tinnitus, while not all models incorporate the notion of direct involvement of peripheral parts of the auditory pathway.

In the peripheral model of tinnitus, alterations in peripheral anatomy (i.e., loss of cochlear hair cells (Eggermont and Roberts, 2004)) as well as (related) alterations of peripheral physiology (i.e., increased cochlear activity (Mulders and Robertson, 2009)) are both causes and key players in maintenance of the subjective tinnitus perception. The peripheral model is able to explain the related increased neuronal firing between peripheral (here:cochlear) and central parts of the auditory pathway (Mulders and Robertson, 2009) as well as decrease of the tinnitus sound after auditory nerve section in some sufferers (House and Brackmann, 1981). In contrast to the experimental data (partly) supporting peripheral cochlear activity as the indispensable generator of the tinnitus signal, further experimental data does not support this model, as Schaette et al. (2012) were able to show that simulated unilateral hearing loss induces a reversible sensation of a phantom sound

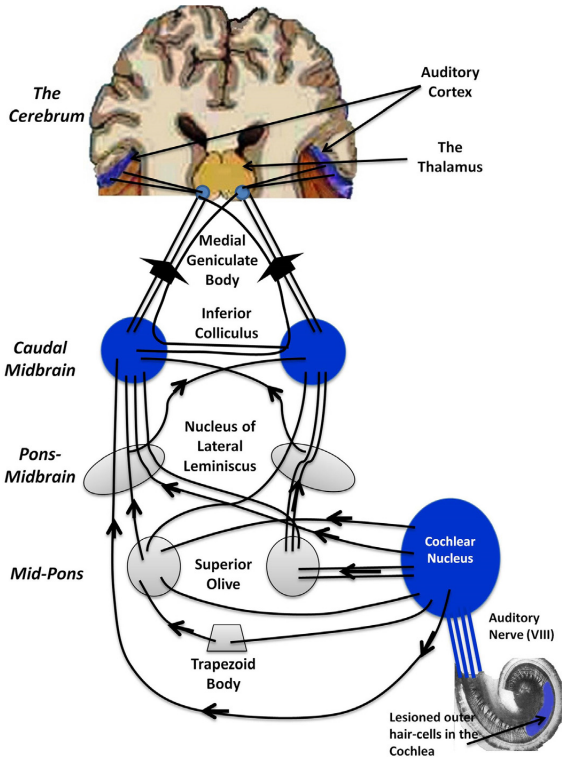

Figure 1.1: Pathways and structures involved in tinnitus. Schematic of the ascending auditory pathways showing structures involved in tinnitus, from the cochlea to the auditory cortex in the brain. Human, but mainly animal studies of tinnitus have revealed increase in spontaneous activity, burst firing, and synchronous discharges at various stages of this pathway following lesions of the hair cells in the cochlea. These areas with structural and functional change in tinnitus are shown in blue. Adapted from Adjamian et al. (2014).

in healthy subjects. Furthermore, the model is not supported by the observation in House and Brackmann (1981) that 55% of the patients with auditory nerve section reported no change or worsening of their tinnitus symptoms.

Moving up the auditory pathway, various models propose that tinnitus is generated and maintained by subcortical hyperactivity, which is then relayed to the auditory cortex. This hyperactivity is reflected by increased 'central gain' (Noreña, 2011; Schaette and McAlpine, 2011; Zeng, 2013) and may be differentiated as increased central noise in the case of tinnitus in contrast to increased nonlinear gain in hyperacusis (Zeng, 2013). According to this model, the increase in central gain or noise may be observed in all nodes of the auditory pathway and does not necessarily rely on increased cochlear activity (see

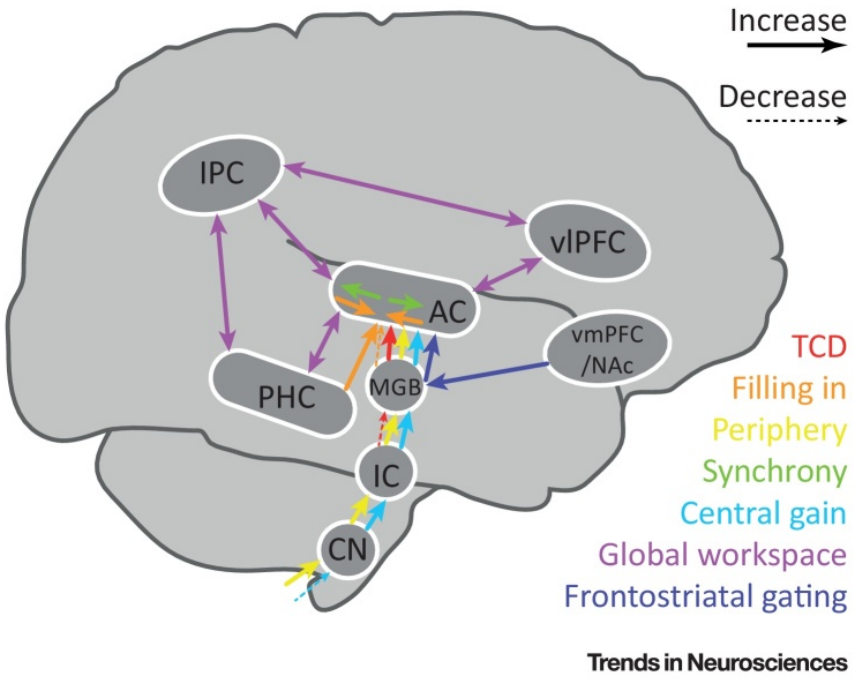

Figure 1.2: Schematic of altered inter-areal inputs in existing models of tinnitus generation (Sedley et al., 2016). AC = auditory cortex. CN = cochlear nucleus. IC = inferior colliculus. IPC = inferior parietal cortex. MGB = medial geniculate body. NAc = nucleus accumbens. PHC = parahippocampus. vlPFC = ventrolateral prefrontal cortex. vmPFC = ventromedial prefrontal cortex.

figure 1.2). Related to this concept, neural synchrony models (Seki and Eggermont, 2003; Noreña and Eggermont, 2003) postulate that both spontaneous firing rates (SFR) and neural synchrony increase following frequency-specific acoustic (i.e., noise) trauma in the regions of tonotopic map reorganization in the primary auditory cortex (PAC, here: in cats). If and how exactly this increase in neural activity/synchrony contributes to the perception of the phantom sound in humans (e.g., (Mühlnickel et al., 1998) is still discussed and neural correlates of frequency-specific changes in PAC are not easily accessible due to the low spatial resolution of standard electrophysiological devices applied extracranially (EEG, MEG). Moreover,the general discussion about a linear frequency gradient in the tonotopy of human PAC in general and in tinnitus in particular further complicates the debate (Langers, 2014). Yet, increased high frequency activity in PAC may very well be related to these neural synchrony models as seen in increases of gamma oscillatory power in some studies (e.g., (Weisz et al., 2007; Ashton et al., 2007; van der Loo et al.,

2009) while other studies did not find any significant alterations in the gamma band (e.g., (Balkenhol et al., 2013; Zobay et al., 2015; Pierzycki et al., 2016).

The model of frontostriatal gating (Rauschecker et al., 2010, 2015) is worth mentioning at this point due to its core assumption that subcortical activity or noise reflecting the tinnitus signal is not properly suppressed, mostly via interneurons in the thalamic reticular nucleus, in the thalamus. This putative 'noise canceling' mechanism is normally mediated by cortical medial frontal or subcallosal structures, thus cortical 'control', which is believed to be impaired in tinnitus sufferers. Functional MRI data supporting this model was presented by the same group (Leaver et al., 2011) with most activity in reaction to a sound matched to the tinnitus in the regions of the nucleus accumbens (NAc), a region highly interconnected to ventromedial prefrontal cortex (vmPFC) and theorized to be involved in noise canceling in the thalamo-cortical relay. Looking at magneto- and electroencephalographic (MEEG) methodologies, several EEG studies performed by a different group could show correlates between oscillatory power and connectivity in the alpha or beta band and tinnitus distress or general mood in these regions (De Ridder et al., 2014). As of today, only one group (study 1 of this thesis) could partly replicate the finding of altered structural morphology in vmPFC (Meyer et al., 2016). Unlike the functional MEEG data or the theorized interaction between mood (anxiety and depression) and tinnitus, this data showed a decrease in cortical thickness in the subcallosal area in vmPFC as a function of tinnitus duration solely, whereas distress or depression scores had no correlate of structural alteration in this region.

In the model of thalamocortical dysrhythmia (TCD) established in the theoretical context of neurological and neuropsychiatric diseases, enhanced cortical high frequency activity also plays a critical role. In this model, deafferentation (as in neurogenic pain or tinnitus) leads to a bottom-up alteration of thalamic theta oscillations (4-8 Hz) which in consequence can trigger high frequency activity via activation of thalamocortical relays (harmonic 'edge effect'). In tinnitus, altered cortical theta and gamma oscillations may be measured as a consequence of this dysrhytmia. Increases in theta band power were indeed observed in a study comparing tinnitus sufferers with healthy controls (Moazami-Goudarzi et al., 2010), but also decreases in an other study (Vanneste et al., 2010). Furthermore, theta band power was positively correlated with tinnitus distress in one study (Balkenhol et al., 2013) and to temporary tinnitus intensity (in a tinnitus suppression or

residual inhibition (RI) paradigm (Sedley et al., 2012)). All in all, theta band power alterations as theorized in the TCD model were far from ubiquitous in experimental neurophysiological studies, especially not in studies with group comparisons between tinnitus sufferers and healthy controls. This may be explained in shifts of the theta band power to different 'carrier waves' like higher alpha band oscillations in tinnitus (De Ridder et al., 2015). Alpha band power decreases in tinnitus sufferers compared to healthy controls (Weisz et al., 2005, 2007; Schlee et al., 2014) were theorized to be dependent on deafferentation and related oscillatory shifts in the TCD model leading to decreased cortical alpha band power. Yet, also in this frequency band results diverge with reported null findings from different groups (Balkenhol et al., 2013; Zobay et al., 2015; Ashton et al., 2007). Regarding high frequency gamma band oscillations, the increase in gamma frequencies theorized by the TCD is somewhat more consistent (Ashton et al., 2007; Weisz et al., 2007; van der Loo et al., 2009). Recent insights into gamma brain connectivity (Schlee et al., 2009a) and a possible shift of the global power spectrum from the normal 1/f pink noise distribution mirroring well-balanced brain networks to abnormal $1/f^0$ white noise distribution with increases in high frequency power typical to neurological or neuropsychiatric symptoms (Mohan et al., 2016) add further value to the TCD model. Beyond that, gamma power and connectivity increases also fit other models like the neural synchrony model introduced above, filling-in models where lacking peripheral auditory input is replaced by recruitment of (nearby) cortical resources (De Ridder et al., 2014; Roberts et al., 2013), and global workspace models (De Ridder et al., 2014). According to this latter model, the (final) perception of the tinnitus sound is reflected by conglomerate high frequency (i.e., gamma) activity of various network hubs in the brain contributing differential aspects of perception, attention, emotion and cognition (De Ridder et al., 2014). These overlapping general but also tinnitus-specific networks may then very well explain further aspects and the individual extent of the tinnitus suffering (e.g., tinnitus loudness, distress, or maladaptive coping) as well as differential manifestations of pathogenesis or chronification states. Bridging between the TCD and the global workspace model, De Ridder et al. (2015) incorporated TCD in the assembly of concurrent tinnitus models with ease.

Taken together, findings supporting the models are diverging which comes as no surprise given the inherent heterogeneity of tinnitus. Beyond that, no existing study prop-

erly replicated a former study with identical study samples and methodologies, which increases the uncertainty in many proposed models and theoretical frameworks. An attempt towards a reconciliation of results has been recently performed by our group with mixed results (Meyer et al., 2017). We could replicate tinnitus distress specific alterations in beta bands but were not able to re-instantiate the exact cortical location using the same EEG methodologies as in the former study (Joos et al., 2012). This generally inconsistent data situation is not only hampering basic research in tinnitus but also relativizes putative neuromodulatory methods (e.g., cortical auditory gamma band power) as targets for possible efficient therapeutic interventions. It is therefore deemed of utmost importance to switch gears downwards and proceed carefully with replication of current data as well as probing the different models along internationally established guidelines by experts in the(ir) field. In the case of MEEG methods some standardization efforts were already undertaken (http://tinnet.tinnitusresearch.net/images/Standardisation_Report_V5.pdf) and are now, hopefully, deployed into the study workflows of different groups in tinnitus research.

At this point, basic modeling of tinnitus in mostly subcortical parts of the auditory pathway is abandoned and a return to the tiers of neuropsychological expertise central to the thesis at hand, namely structural alterations of the cortex and acoustic stimulation with a novel stimuli class aiming at normalization of cortical activity in tinnitus, is undertaken. Nevertheless, the given insights about the complex interplay of the various elements of the auditory pathway between the inner ear and the brain are recommended to be kept in mind for the remainder of this thesis, especially for the acoustic stimulation studies.

1.2 Cortical Structural Alterations Related to Tinnitus

1.2.1 Methods

Structural alterations related to tinnitus are of high interest given that the understanding of the brain imprint of tinnitus chronification and maintenance not only is deemed fruitful for basic research and modeling, but also to identify possible targets for neuromodulatory, behavioral and eventually acoustic interventions. Yet, given the omnipresent heterogeneity of tinnitus and related study findings, it is not surprising that the branch of neuroanatomic studies in tinnitus does also suffer from the plague of partly irreconcilable results.

First, the most common methodologies, also applied in the tinnitus literature reviewed here, are introduced. As the thesis did not consider the tracking of white matter fiber tracts (i.e., diffusion tensor imaging (DTI), (Assaf and Pasternak, 2008)), the reported methods are restrained to estimates of gray matter (GM) using either voxel-based morphometry (VBM, (Ashburner and Friston, 2000)) or surface-based morphometry (SBM, (Fischl et al., 1999a, 2004a; Desikan et al., 2006; Destrieux et al., 2010).

VBM basically takes the cubic voxels of an MRI image series, registers them into a common space, and finally smoothes it so that every resulting voxel reflects GM density of itself and neighboring voxels. The resulting voxel matrices can be compared between groups, or conditions, or they can be modelled with more complex statistics (statistical parametric mapping (SPM) (Friston et al., 1994)), resulting in clusters of focal differences of GM. Given the resulting differences in three dimensional voxel clusters, VBM exclusively provides volumetric measures of cortical GM.

SBM, on the other hand, is a fully automated observer-independent iterative method segmenting cortical tissues and finally extracting the cortical GM layer with sub-voxel (or -millimeter) precision. In comparison to VBM, it is very costly when it comes to computing resources as a single brain cortical surface model can take up to 24 hours on a modern single core computer. Yet this computational investment pays off as very precise and accurate outputs are generated. After registration, inflation and smoothing, a final two dimensional cortical map is generated and ready for further analyses applying similar statistical methods as VBM. Notably, SBM produces outputs of cortical thickness (CT), the distance between white matter and pial surface (i.e., cortical GM) at each vertex point of the two dimensional mesh grid (in mm), a normalized value for the cortical surface area

(CSA) perpendicular to thickness, and finally a volumetric parameter (CV) comparable to the volume of VBM, which is the arithmetic product of thickness and area. Beyond these volume-forming, partly independent, parameters, SBM is able to produce various measures of cortical folding like a gyrification index or mean curvature to complement the distinct analysis of cortical morphology.

Generally, the methods produce similar outputs for cortical GM volume whereas SBM can be regarded as superior, especially on the cortical level, which the method is specialized for. First, cortical surface reconstruction in SBM is performed on individual brains generating individual surface models with exact folding patterns which are projected into common registration spaces (e.g., for statistical analysis) at very late stages of the computational pipeline. VBM, on the other hand, does not account for these individual nuances and segmentation of GM is only performed after registration into common space, which is seen as a major drawback of the method (Mietchen and Gaser, 2009). Furthermore, VBM has to assume partial volume effects (i.e., due to borders between white and GM not following the borders of the predefined voxels) and only CV can be measured directly (see figure 1 of Winkler et al. (2010)). There may be advantages in usability and documentation of the widely accepted VBM method, while SBM has just recently gained traction coinciding with better documentation and the inception of graphical user interfaces (GUI) for result viewing or simple statistics. Looking at the issues of validity and scientific interpretability, SBM with its more sophisticated cortical surface reconstruction pipeline (yet massively increased computational efforts) and wider array of partly independent morphological measures certainly outperforms VBM in many tasks. SBM methods have been validated against classical histological analysis (Rosas et al., 2002) and manual measurements of cortical structures (Cardinale et al., 2014; Kuperberg et al., 2003; Salat et al., 2004). Furthermore the various measures have been shown to be reliable even with different smoothing kernel widths (Liem et al., 2015). CT and CSA can be considered as independent parameters of GM where CT is rather a reflection of plasticity related to pathologies, learning, or aging over the lifespan, whereas CSA may rather mostly be determined during early brain developmental phases (Storsve et al., 2014; Panizzon et al., 2009).

Hitherto, SBM is underrepresented in tinnitus research with just one study applying this method alongside VBM to probe neuroanatomy in tinnitus (Leaver et al., 2012). Mean-

while, during publication process of our study (study 1 covered in this thesis), two additional groups published neuroanatomical tinnitus data analyzed with SBM (Allan et al., 2016; Yoo et al., 2016). In the following paragraph and overview over concurrent SBM and VBM studies in tinnitus are given and respective open research questions derived.

1.2.2 Heterogeneous Findings in Tinnitus-Related Neuroanatomy

The first study of tinnitus-related neuroanatomy using VBM was performed by Mühlau et al. (2006) comparing 28 TI with healthy controls matched for hearing (normal hearing in both groups), age, and gender. Results indicated alterations in thalamus (i.e., increase in GM, region of interest (ROI) analysis) and vmPFC (i.e., decrease in GM) but no alterations in (primary) auditory regions. Yet, the resulting loci certainly inspired or gave rise to the frontostriatal gating model proposed by Rauschecker et al. (2010). Landgrebe et al. (2009) then applied identical sample sizes, matching strategies, scanner protocols, and analysis software resulting in differential findings with GM decreases in right IC and left hippocampus according to ROI analyses. In the same year, a study was published using a method similar to SBM where the Heschl gyrus (HG) of individuals was mapped onto a surface resulting in differential sizes, gyrations, and duplication of HG (Schneider et al., 2009). HG volumes were overall reduced in TI compared to the control group and it was concluded that smaller volumes in auditory regions may point to a specific vulnerability to tinnitus. Leaver et al. (2011) replicated the findings of Mühlau et al. (2006) with a smaller sample size controlling for the covariates of age and gender. Furthermore, in this study, a 3T (three tesla) scanner was applied and the data was analyzed using further developed versions of the VBM/SPM software suite. Notably, the findings fitted the proposed model of frontostriatal gating by Rauschecker et al. (2010) to the most part. A further study, with even smaller sample size (i.e., eight patients vs. seven controls vs. eleven controls without hearing loss), all matched for age and gender, was the first one to report null findings as no differences were found for tinnitus but for hearing loss (Husain et al., 2011). Diesch et al. (2012) exclusively focused on alterations in the corpus callosum (CC) exclusively and found respective larger CC volumes in TI (n=63) compared to controls (n=42). The effect was gender-dependent and it was theorized that the increased CC volume is indicative of a feedback loop between the bi-hemispheric parts of auditory cortex. As mentioned in the last paragraph, the study of Leaver et al. (2012) was the first to apply SBM with the

FreeSurfer software (http://freesurfer.net/) alongside VBM comparing 23 TI vs. 21 controls matched for age and gender. The main findings here were GM decreases in vmPFC, dorsomedial prefrontal cortex (dmPFC), and supramarginal gyrus (SMG). These VBM findings were then partly extended with SBM measures with decreases in CT and CSA in vmPFC, and increased folding reflected by the curvature parameter in SMG and dmPFC. In addition, they reported a decrease in thickness in subgenual anterior cingulate cortex (sgACC) related to higher combined anxiety and depression scores further contributing data to the frontostriatal gating hypothesis (here: interaction with depression or serotonin systems). Boyen et al. (2013) again applied VBM analysis on three groups (31 TI, 16 controls with and 24 without hearing loss) comparable for gender but not age distribution. Results indicated an increase in GM in left PAC in a ROI analysis contrasting TI with all of the controls. Finally, Aldhafeeri et al. (2012) performed another neuroanatomic study comparing TI with healthy controls. 14 TI were contrasted to an equal amount of healthy controls matched for age, gender and hearing loss using similar methods as Schneider et al. (2009) but on different ROIs. They found GM decreases in PFC, cingulate and temporal cortex.

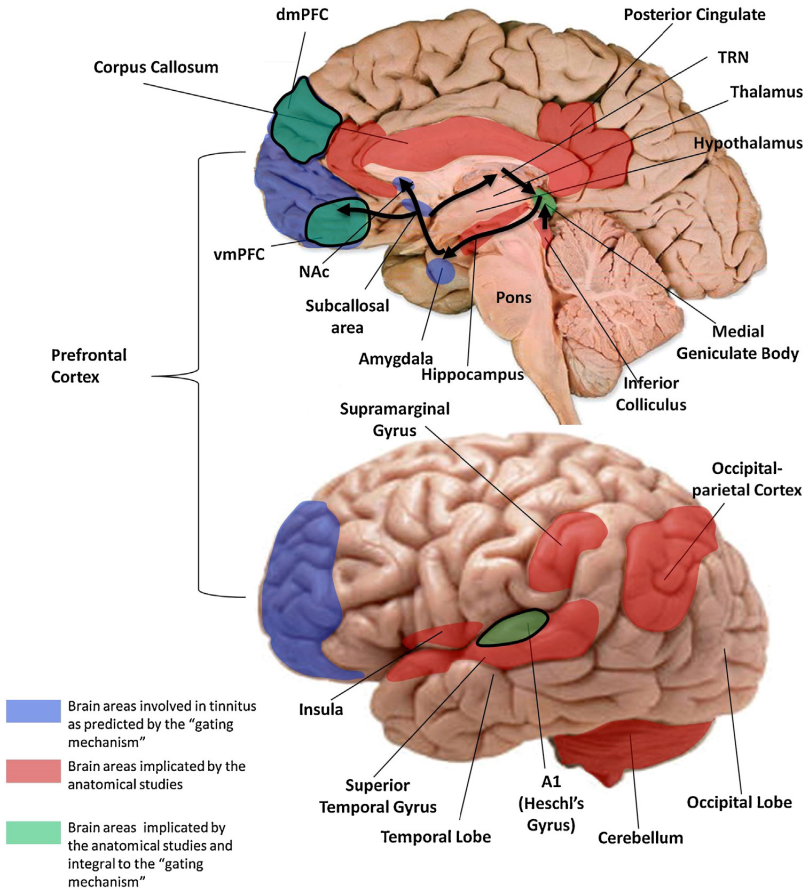

Figure 1.3: Neuroanatomical changes in tinnitus. Brain areas proposed to be involved in the gating mechanism (blue) and those discovered by anatomical MRI studies of tinnitus. Areas common to both are shown in green. Note that vmPFC and dmPFC were reported as effects of hearing loss rather than tinnitus (Melcher et al., 2013). The corona radiata and the longitudinal fasciculus are not shown. The arrows represent the flow of neural activity arriving at the IC and MGN and relayed to the primary auditory cortex for perception. The signal is then sent via the amygdala to the subcallosal region and the NAc for evaluation of emotional content. From here, the reticular nucleus of the thalamus receives an excitatory feedback, which inhibits the section of the MGN corresponding to the tinnitus sound (see Rauschecker et al. (2010)). Adapted from Adjamian et al. (2014).

The introduced studies can be regarded as the first wave of neuroanatomy studies in tinnitus mostly comparing TI with controls with relatively small samples sizes and the application of VBM. In the following, a new wave of neuroanatomical studies is presented

which exhibit consistent matching strategies, large samples, up to date analytical tools, whole-brain instead of ROI analyses, and critical input in form of a null finding. Melcher et al. (2013) chimed into the ongoing discussion about the role of sgACC and hearing loss and its putative neuroanatomical correlates by presenting null findings for the group contrast between well-matched TI and controls. In order to grasp this null finding and understand the role sgACC in tinnitus, the authors performed ancillary analyses where they found negative correlations between hearing loss in frequencies beyond the scale of normal audiometry (i.e., above 8000 Hz) and GM in ventral posterior cingulate cortex, dmPFC, and sgACC. It was thus concluded that alterations in these regions, especially in sgACC central to the frontostriatal gating model, were not related to tinnitus but rather to high frequency hearing loss above the standard audiogram.

In the same year, the largest VBM study to date was published sporting a total sample size of 333 TI (Schecklmann et al., 2013). The authors refrained from a control group due to suboptimal matching conditions and applied an analysis of covariance (ANCOVA) between tinnitus distress as measured by the Tinnitus Questionnaire (TQ, (Goebel and Hiller, 1994)) and GM alterations in a whole-brain analysis corrected for a variety of nuisance factors namely age, sex, hearing loss, tinnitus duration, and tinnitus laterality. Results showed that the bilateral auditory cortex is implicated in tinnitus distress with the temporal clusters being negatively correlated to tinnitus distress. This results goes in line with the previous observation of Schneider et al. (2009) and Aldhafeeri et al. (2012), especially when TQ scores are regarded as proxies of general tinnitus strength (thus cautiously bridging between between- and within-subjects designs). On the other hand, with no GM alterations observed in relation to hearing loss as measured with standard audiometry up to 8000 Hz, the issue of GM alterations related to high frequency hearing loss raised by Melcher et al. (2013) could not be convincingly tackled. Yet, given the large sample size (at least five to ten times larger than in previous studies) and the plethora of (co)variables included in a careful analysis including cross-validation in a smaller subsample, the results convincingly point to a relation between reduced GM (volume) and tinnitus.

In the spirit of applying general linear models (GLM) and large sample sizes (Schecklmann et al., 2013), Vanneste et al. (2015) performed a correlational study between tinnitus parameters and VBM as well as quantitative EEG (qEEG) measures with a sample of 154

TI. Interestingly, in this study, which is applying similar methods but a different sample, the negative correlation between tinnitus distress and temporal auditory GM could not be replicated while the analysis produced significant decreases of GM in auditory cortex and the thalamus. Further tinnitus parameters like tinnitus duration, intensity levels, type (i.e., pure tone vs. noise-like tinnitus), or lateralization did not produce significant (at best at levels uncorrected for multiple comparisons $p<0.001$) or meaningful results. Beyond that, authors reported the complete absence of any relation between VBM and qEEG failing to link structural to functional changes as elicited by resting state EEG. The conclusion was therefore disillusioning with no results surviving strict correction for multiple comparisons except a cluster in the cerebellum which led to the conclusion that VBM may not be sensitive enough to discover changes in GM related to tinnitus.

To close, two final studies are introduced which (unfortunately) coincided with the publication of study 1 of this thesis, limiting the dramaturgy of the passage at hand. However, for the sake of completeness of this overview and since they have only minor influence on the research question in this thesis, they are still valid, relevant, and introduced at this point.

Yoo et al. (2016) investigated the relationships between cortical thickness (CT as elicited by FreeSurfer) and behavioral measures of aging, tinnitus loudness, tinnitus duration, tinnitus distress, and hearing loss in a sample of 127 TI. Again, results were indicative of weak to null effects, with only thalamic GM volume (produced by the automated subcortical stream of FreeSurfer comparable to VBM volume estimates) being negatively correlated to tinnitus distress and loudness in simple bivariate correlational analysis. More sophisticated statistical models controlling for covariates could only produce results for hearing loss and widespread decrease of CT in frontal regions related to age, which is a well-documented observation (Salat et al., 2004). Notably, in the article only changes in CT were reported in the article and a comparison to the VBM methodology was not performed.

Finally, a further group embarked on the endeavor to probe neuroanatomy for tinnitus-specific alterations (Allan et al., 2016). As to this date, results still were far from consistent across studies and also larger sample sizes in combination with within-subjects designs could not convincingly address the persisting issues in this line of research. These observations led Martin Schecklmann, author of Schecklmann et al. (2013), to a statement

that every further study may only be another “coffin nail” to the casket of this line of research (personal communication). Unsurprisingly, again, the analysis of 128 TI produced only robust effects of GM alterations for age, sex, and hearing loss while tinnitus-specific alterations were reported but only cautiously interpreted. There were decreases in GM (CT) in the contrast between TI and healthy controls and also decreases in CT, CSA, and CV in relation to tinnitus severity as elicited by the Tinnitus Handicap Inventory (Newman et al., 1996) in auditory cortex, precuneus, and middle frontal regions. Of special note, differential alterations were reported as CT was also positively correlated to tinnitus severity in middle temporal regions in contrast to the decreases in or near PAC. While the results were again not really convincing, which led the authors to the conclusion that much has to be invested still, especially in matching of subgroups in large study samples, the impression emerges that SBM may be more sensitive to subtle tinnitus-related changes in macroscopic brain anatomy than the standard VBM approach.

1.2.3 Future Directions and Open Questions

As seen and iterated in the previous paragraph, the reasons for the heterogeneous and inconsistent findings across studies are manifold (Adjamian et al., 2014): First and foremost, the overarching inherent heterogeneity of the tinnitus suffering still seems to be a very limiting factor to produce consistent study results reflected in the large absence of replicated data throughout neuroanatomical literature in tinnitus. Furthermore, even with ongoing research to get a handle on this elusive heterogeneity, many latent variables and concepts might not have been discovered yet. Besides the often discussed issue of proper sample stratification, a discussion has been initiated recently regarding sampling strategies ranging from clinical to more casual approaches, like internet self-help forums (Probst et al., 2017). While the study could unfortunately not compare main clinical parameters like tinnitus distress, it is deemed fruitful to sample across the whole range of approaches to obtain larger samples with an inherent wider distribution of symptoms and to get hold of (sub)acute TI to study early phases of tinnitus chronification. Returning to aspects specific to the neuroanatomical studies, Adjamian et al. (2014) propose that at least the core set of age, sex, and hearing loss has to be carefully considered for proper matching of the groups or large scale correlational analyses. Furthermore, as introduced in the newer studies (Schecklmann et al., 2013; Vanneste et al.,

2015; Yoo et al., 2016; Allan et al., 2016) further tinnitus parameters like distress, duration, loudness, time aware of tinnitus (usually in percent), type, and laterality should be considered and carefully checked in these analyses. The use of different methodologies and also MRI scanner technology or protocols further complicates the emergence of consistent interpretable results in these studies (Adjamian et al., 2014). On the one hand, scanners and their protocols certainly have evolved in the last ten years during which these studies were performed so that scanners with stronger 3 Tesla magnetic fields are more widely used increasing the precision of acquired MRI image sequences. On the other hand, especially in regard to VBM, many (partly fundamental) software updates have been performed which certainly influenced the results (for a comprehensive overview of used VBM version the interested reader is referred to table 1 in Schecklmann et al. (2013)). Disregarding software updates, large variability can still be introduced by improper use or intransparent reporting of analysis parameters as criticized in Adjamian et al. (2014) with a reference to standardized analysis and reporting guidelines for VBM (Ridgway et al., 2008). Fortunately, these methodological issues are dealt with and standards established within the MRI subgroup of the working group 'neuroimaging' of the TINNET research initiative (http://tinnet.tinnitusresearch.net/index.php/2015-10-29-10-22-16/wg-3-neuroimaging). In conclusion, to improve both the reliability and validity of the results these recommendations have to be considered in all future studies of neuroanatomy but also all other studies in tinnitus. Future studies should therefore integrate a transparent analysis pipeline from sample description to properly modeled statistical analysis on the assumption-free whole-brain level followed by post hoc ROI analyses if indicated. Results should be corrected for multiple comparisons where appropriate and they should generally be reported transparently, including uncorrected results. As uncorrected results may not be the gold standard in concurrent neuroscience, they may be insightful hints for tinnitus research given the observed overall weak effect sizes for tinnitus-specific GM alterations. Certainly, these weak effects could be overcome by simply increasing sample sizes, which is a tedious endeavor on its own, and may only be resolved by pooling of MRI data in large databases. The 'second wave' of neuroanatomical studies in tinnitus already conducted and reported their studies in that spirit with maximally large samples (Schecklmann et al., 2013; Vanneste et al., 2015; Yoo et al., 2016; Allan et al., 2016). Furthermore, a marked shift is discernible between clas-

sical case-control studies to a more in-depth analysis within TI. This approach may partly reduce problems inherent to the heterogeneity problem and may ease the much needed subtypization efforts for the tinnitus population (Meyer et al., 2014b, 2017).

Looking at neuroanatomical alterations in relation to tinnitus, several questions remain unanswered.

First, the question whether auditory cortex morphology is altered either as a consequence or prerequisite of tinnitus is still unanswered and of utmost interest given the central role of auditory cortex in various tinnitus models.

Second, as already partly covered in the first question, it remains to be determined whether tinnitus rather emerges as a consequence of smaller GM volumes in auditory regions or if alterations in these regions (Schneider et al., 2009) are a consequence of (ongoing) tinnitus chronification therefore hinting at tinnitus-specific neuroplasticity.

Third and building on the second question, are these putative alterations related to the differential neuroanatomical traits of CT (reflecting life-time maturation, aging, and plasticity) and CSA (possibly reflecting (genetic) predispositions determined early in cortical development) (Storsve et al., 2014; Panizzon et al., 2009))?

Fourth, how could SBM results, especially CT and CSA, extend the insights of VBM CV results in other than auditory regions?

Finally, how are CT and CSA related to both tinnitus distress, coding the severity and intensity of the tinnitus suffering, and tinnitus duration, a marker for tinnitus chronification and possible correlate of tinnitus-related neuroplasticity?

The questions that have been arisen here, therefore, led to the inception of study 1 reported in this thesis.

1.3 Acoustic Stimulation in Tinnitus

Acoustic stimulation or related concepts have a long tradition in tinnitus therapy or management (Hoare et al., 2014). Given that tinnitus has long been conceptualized as a disease of the ear or other peripheral auditory organs, the approach, besides medical surgical treatments and pharmacology, to reinstantiate auditory aspects of tinnitus via acoustic stimulation can be well grasped from our modern, more encompassing view of the tinnitus phenomenon. As a side note, it took a seminal farsighted theoretical article by Jastreboff (1990) to usher in a new age of tinnitus understanding beyond a 'mere disease of the ear'.

The first steps in acoustic stimulation where not designed to treat the symptom but rather served as tools of basic audiological research to measure and probe tinnitus. This was done by performing audiometry and then matching the frequency of tinnitus (which mostly is manifest as a pure tone or a noise with a dominant center frequency) to study the interrelation between matched tinnitus frequency and configuration of the hearing loss (e.g., (Wegel, 1931; Fowler, 1940)). As a consequence of these studies, the phenomenon of tinnitus masking was discovered and served as the basis for respective experimental studies (Feldmann, 1969a,b, 1971; Vernon, 1977). Naturally, the approach showing efficacy sparked the interest of audiologists and clinicians desperately looking for any effective treatment for the phantom sound. Beyond that and of utmost interest, in these earliest studies an after-effect of tinnitus masking was observed in that the internal tinnitus sensation remained suppressed after offset of the external masking stimuli (Feldmann, 1969a,b, 1971). This effect was termed 'residual inhibition' (RI) and furthermore serendipitously embraced by the community as reflected by a statement in the discussion of Vernon (1977): "When masking of tinnitus is effective it produces a suppression of the tinnitus which extends beyond the duration of the masker. This is termed residual inhibition, a matter about which we need to know a great deal more." Masking of tinnitus can either be 'total' so that the stimulation level of the masker is sufficient to completely abolish the percept of tinnitus or it can be 'partial' so that tinnitus is still audible to some degree (Hoare et al., 2014). In reality, stimulation levels have to be (individually) adjusted on the continuum between total and partial masking to reach the sweet spot for optimal tinnitus suppression or relief. This is still valid in concurrent (audiological) practice where every hearing aid with or without maskers, soothing, or distractor sounds, has to be fitted individually to achieve the best results (Henry et al., 2005a,b). The point

on this continuum where tinnitus is completely masked and therefore inaudible is called minimum masking level (MML) (Terry et al., 1983). Most importantly, stimulation levels have to be carefully obtained and possibly adjusted throughout the therapeutic process to ensure efficacy. As mentioned before, masking or generally auditory therapies (excluding the newer approaches building on recent neurophysiological models of tinnitus focusing on central changes (i.e., map plasticity and lateral inhibition (Noreña, 2015)), besides direct audiological and physiological effects, are also effective on an indirect psychological level in providing relief from the tantalizing phantom sound. Taken together, masking has therapeutic value for tinnitus and it is still used widely, ideally combined with counseling (Hazell et al., 1985; Baguley et al., 2013).

Currently, besides masking and (related) RI, two approaches building on central maladaptive plasticity as well as lateral inhibition are extensively tested (Okamoto et al., 2010; Tass et al., 2012) and worth mentioning due to their prominence as well as putative efficacy. Both approaches are based on considerations about dysfunctional lateral inhibition of neighboring neuron populations in PAC following deafferantation of peripheral input (i.e., loss of cochlear hair cells as a marker of any hearing loss) and subsequent reorganization of the tonotopic map (Mühlnickel et al., 1998; Seki and Eggermont, 2003; Eggermont and Roberts, 2004). Put simply, the aim of the approaches is to reinstantiate normal lateral inhibition in PAC by stimulating with sounds around the actual tinnitus frequency, thus stimulating lateral inhibition towards the deafferented regions at or near the tinnitus frequency, which in turn should diminish the phantom percept as the aberrant central hyperactivity is then normalized again. Schematic depictions of the frequency distribution of the sounds of the two approaches are evident from figure 1.5, panels c and d. Notably, Pantev et al. (2012) apply a notch filter (usually 1 octave in width) around the tinnitus frequency in music stimuli so that the remaining, unfiltered frequencies can gradually reinstantiate the dysfunctional lateral inhibition. Tass et al. (2012) rely on presenting single tones at various frequencies in the frequency spectrum one octave or more around the tinnitus frequency in a randomized fashion to obtain similar results. As introduced above, both approaches, especially the one of Pantev et al. (2012), are followed with great interest and partly already commercialized (http://www.tinnitracks.com/en), Yet, there is some debate about the efficacy of the approach by Tass et al. (2012) (Wegger et al., 2017) contrasted by respectable neuroscientific data related to their acoustic stimulation by the

authoring group (Adamchic et al., 2014, 2017). On the contrary, a manifold of other approaches is deliberately left out in this thesis due to the non-existence or dubious nature of related scientific publications, lacking linkage to established methods and valid models of tinnitus, and suspected mere commercial interests. Furthermore, fostering this impression, many hyped approaches vanished shortly after the first critical evaluations. The interested reader is referred to a comprehensive overview of the approaches in acoustic stimulation in tinnitus (Hoare et al., 2014) or to a more theoretical update on some selected relevant approaches (Noreña, 2015; Adamchic et al., 2012).

Coming back to RI, a concept central for the studies of acoustic stimulation in tinnitus within this thesis, has been shown to be effective when produced by broadband noise masking and accompanied by counseling in retrospect to several studies (Vernon and Meikle, 2000). A recent study by Roberts et al. (2006) applying masker sounds at high stimulation levels for 30 seconds elaborated on RI effects and found that with increasing center frequency of a band-passed noise both duration and depth of tinnitus suppression increased. Furthermore, they observed a near elimination of the tinnitus sound up to 45 seconds after stimulus offset in about a third of participants. Another key observation in this study was the fact that RI was most efficient when sound stimuli (mainly the center frequency) was near or overlapping the matched tinnitus spectrum (Norena et al., 2002). This may imply that tinnitus suppression works best in frequencies in or around the (matched) tinnitus frequency. Indeed, this observation has received both theoretical (Schaette et al., 2010) as well as empirical backing (Reavis et al., 2012). In a follow-up study, Roberts et al. (2008) further evaluated RI and its relation to the tinnitus spectrum and hearing loss in multi-center cohorts with similar results.

Up until now, the stimuli used to mask or temporarily suppress tinnitus were broadband (i.e., white noise), narrow-band noises (i.e., bandpass-filtered to some degree around a center frequency), or pure tones. These stimuli are continuous in nature with no temporal modulations except psychoacoustically indiscernible fast random modulations of noise sounds. Besides the two approaches building on the reversal of central maladaptive plasticity through lateral inhibition (Pantev et al., 2012; Tass et al., 2012) in which temporal modulations play a subordinate role (see figure 1.5. panel d and e), no study specifically looked at the role temporal modulations may play in tinnitus masking or suppression. These modulations could be regarded as the third parametric dimension which may be

manipulated in acoustic stimulation of tinnitus besides the frequency (distribution, see figure 1.5) and the stimulation level of the sounds.

Deriving insights from a study using low frequency electrical stimulation of the cochlea with remarkable effects on tinnitus suppression (Zeng et al., 2011), Reavis et al. (2012) performed a behavioral study testing the influence of various sounds comparing the classical stimuli class (i.e., pure tones, broad- and narrow-band noises) with modulated version of the latter in 20 TI. Concretely, they produced an array of 17 different sounds, namely an amplitude modulated (AM) tone, a frequency modulated (FM) tone, a pure tone (PT), a narrow-band noise (NBN), and white noise. The sounds were, with the exception of white noise, split up in 4 frequency bins between 75-750, 750-1500, 3000-6000, and 6000-9000 Hz, respectively. Each sound was then presented to each participant for three minutes while online (i.e., during stimulation) and offline (i.e., after stimulation offset) tinnitus loudness ratings relative to the initial loudness were assessed every 30 seconds. The modulation rate chosen was 40 Hz building on solid auditory steady state responses (ASSR) with these modulation rates (Ross et al., 2003; Picton et al., 2003) and the stimulation level was set "just softer than his or her tinnitus" (Reavis et al., 2012). Besides continuously indicating their own tinnitus loudness, participants had to simultaneously judge the loudness of the stimulus itself to address the issue of loudness recruitment (Goodwin and Johnson, 1980). Results indicated that all sounds in the lower two frequency bins (75-750 and 750-1500 Hz) exhibited similar tinnitus suppression like the classical masker white noise. In the higher frequency bins, especially in the regions between 6000 and 9000 Hz, all sounds elicited better tinnitus suppression than white noise. However, the contrast between modulated and unmodulated sounds in this frequency bin could only marginally prove the superiority of the modulated stimuli class. Still, with a marginal predicted probability of suppression of 60% of the AM stimuli compared to 45% of the pure tone in the same frequency bin, a pattern of similar if not better suppression of the AM stimuli class compared to PT pendants arises. Notably, the FM stimulus performed second best with a suppression probability of about 50%, yet the authors indicate that the FM stimulus was modulated with just 10% modulation depth compared to 100% modulation depth in the case of the AM stimulus. Looking at the experimental procedure as a whole, one has to imagine how tedious the experimental procedure must have been for the participants, especially in keeping vigilance and motivation up during almost two hours of intense au-

diological testing. Furthermore, both hyperacusis (Baguley, 2003) and potential increase in loudness recruitment (Goodwin and Johnson, 1980) over the span of the experiment introduced further difficulties to the actual experiment and to the concept of the approach. In addition, these difficulties led to the insightful conclusion that "Additionally, our results showed that tinnitus sufferers with less loudness recruitment or hyperacusis were more likely to show tinnitus suppression than those with these clinical symptoms." Reavis et al. (2012). This observation certainly helps in both identifying suitable study samples as well as future treatment populations. Taken together, the results indicate that modulated sounds may indeed surpass classical, unmodulated sounds in their efficacy to suppress tinnitus. Future iterations of this or similar approaches should therefore certainly consider the inclusion of well-matched tinnitus sounds as stimuli (Schaette et al., 2010; Roberts et al., 2006, 2008) and, furthermore, consider longer stimulation phases as no study so far has systematically tested along these lines. In addition, all these studies, while relying mostly on subjective measures and standardized audiological procedures for evaluation of the effects of the stimuli, lack the inclusion of any subjective, reactive psychological measure for the tolerability of the stimuli themselves. With respect to possible therapeutic application of the approach, future studies should consider this aspect.

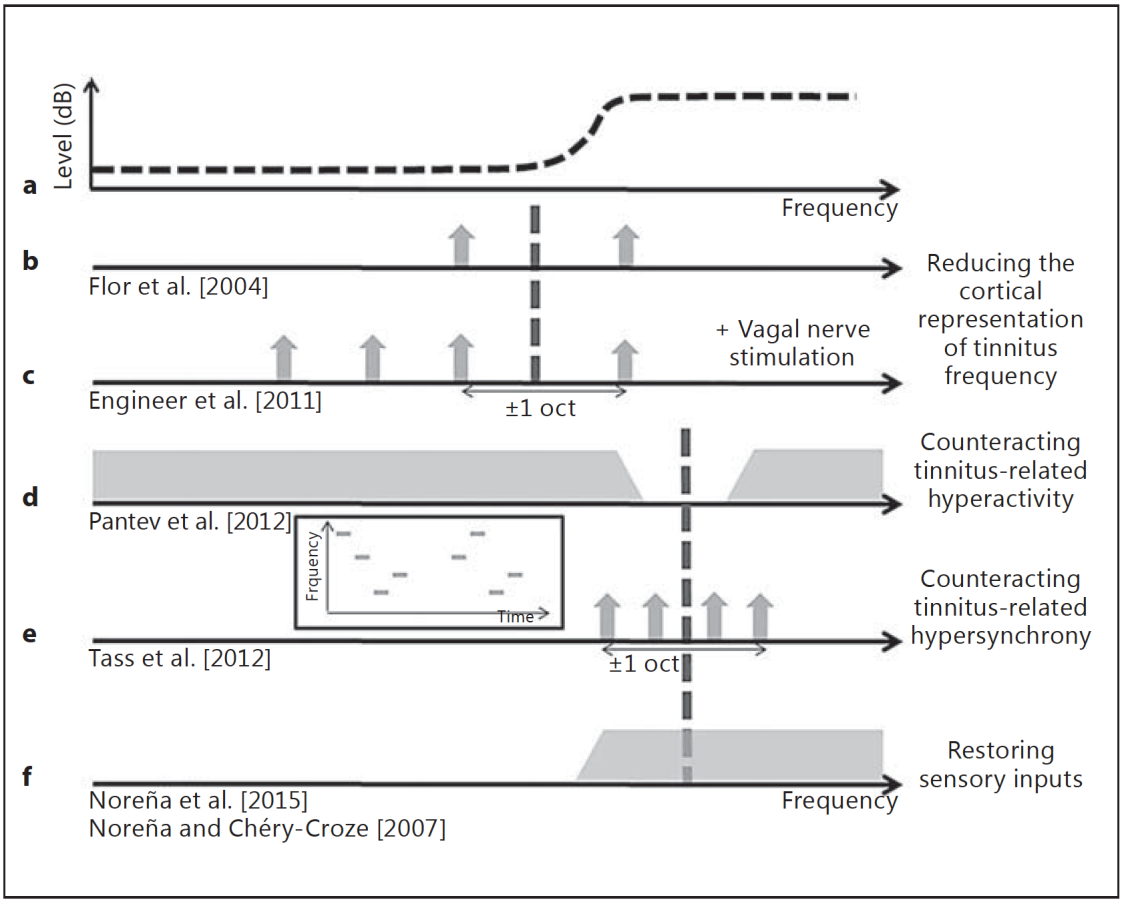

Figure 1.4: Summary of the different acoustic approaches which aim at reversing the tinnitus-related central changes. The schematic at the top (a) shows an idealized audiogram with high-frequency hearing loss. The other rows show the spectrum of the acoustic stimuli developed to reverse the tinnitus-related central changes. (b,c) Spectrum of acoustic stimuli designed to reduce the cortical representation of tinnitus. These approaches assume that tinnitus frequency is overrepresented (i.e. located at the edge frequency of hearing loss – see vertical dashed line). (d) Spectrum of a notched stimulus aimed at suppressing the tinnitus-related activity through lateral inhibition. (e) Spectrum of the acoustic sequence used to reduce cortical hypersynchrony. Inset: the spectrogram of the acoustic sequence is shown as the temporal properties of the acoustic sequence are also critical in this approach. (f) Spectrum of the acoustic stimulus aimed at restoring normal sensory inputs, i.e. those that existed before hearing loss. The vertical dotted lines show where the dominant pitch of tinnitus is expected, i.e. at the edge frequency of hearing loss (b–c) and above this latter frequency (d–f). oct = Octave. Adapted from Noreña (2015).

1.3.1 Methods

To prevent the reader from drowning in a sea of unknown audiological terminology, some key concepts and technologies are introduced at this point with a focus on matching procedures, which are deemed essential to the successful creation and application of acoustic stimulation in tinnitus, given primarily the importance of tinnitus frequency (Schaette et al., 2010) and stimulation level secondarily (Reavis et al., 2012; Roberts et al., 2006, 2008).

In the studies presented in this thesis as well as in comparable studies, audiometry is performed with respective outboard equipment to assess the hearing thresholds at frequencies in the audible spectrum (usually from 125 Hz to 16 kHz) in octave or semi-octave steps. The results are then plotted for each ear separately onto an audiogram and usually, in normal hearing, a linear function of hearing with a downwards slope starting above 8 kHz or more is discernible. At each frequency the respective hearing threshold is represented in dB HL (=hearing loss). DB SL (=sensation level), above this threshold, is the actual difference between e.g. the absolute loudness of a stimulus (measured in dB of physical sound pressure level (dB SPL)) and the db HL at this frequency. These different forms of sound pressure levels or loudness have to be kept in mind for the remainder of this thesis and the included studies. Of special note, stimuli matched to the tinnitus frequency were adjusted to dB HL of the nearest frequency in the audiogram and presented 60 db SL with an upper limit of 80 dB SPL for safety reasons. To ensure naturalistic presentation and full control over the spectral energy of the stimuli, only professional hardware with quasi-linear frequency responses was used (e.g., soundcard, headphones). Finally, careful programming of the stimuli and establishment of the acoustic signal chain further ensured sound presentation without any artifacts (e.g., distortion, clipping).

Tinnitus matching can be regarded, at least, as a subfield on its own in tinnitus research. Concurrently, there are three major methods being studied and applied: The method of adjustment (MOA) procedure enables TI to mostly self-reliantly adjust their tinnitus parameters using controls like sliders, knobs, or touchpads (e.g., (Henry et al., 2004a)). Forced-choice double-staircase (FCDS) approaches make use of bracketing in that TI are continuously presented choices of sounds and requested to choose one of the two sounds which is more similar to their own sound. This procedure continues in lowering the distance in frequency (i.e., bracket) between presented sounds until the best match of tinnitus

has been found (e.g., (Penner and Bilger, 1992)). The pitch likeliness method (PLR) randomly presents reference tones over the audible spectrum which are then rated by TI regarding their likeliness to be identical with their tinnitus sound (rating scale from 0 to 100, (Norena et al., 2002; Basile et al., 2013). Behind all of these methods are some common procedures especially concerning the sequence of matching the various parameters of the subjective tinnitus percept. To avoid loudness recruitment (Goodwin and Johnson, 1980) or, even worse, observed tendencies to confuse tinnitus loudness and frequency (Henry and Meikle, 2000), tinnitus loudness should not be matched before the frequency. There is also debate about whether the frequency should be matched ipsi- or contralateral to the ear where tinnitus or more severe hearing loss is present (Henry and Meikle, 2000). Given the heterogeneity in TI phenotypes performing the matching, a pragmatic procedure of matching in a way which produces best results is certainly most feasible. Octave confusion tests, usually at the end of the procedure, ensure that TI did not match in octaves above or below the actual tinnitus frequency due to the similarity of (musical) tones in the different registers. Finally, test-retest procedures ensure the reliability of the methods which are already implemented in FCDS and PLR through repetitions while MOA lacks this inherent feature. Yet, in comparison, MOA is still able to compete with FCDS (Hauptmann et al., 2016) and comes with the advantage of frequency resolution only limited by the technical equipment, thus enabling more precise matchings, while the other procedures work with predetermined frequencies. Beyond that, the self-reliant nature of the MOA method is deemed to empower TI compared to the passive procedures of FCDS and PLR. For the latter two reasons, the MOA method was used for the two studies presented in this thesis. Concretely, as no specialized equipment is available on the market, custom hard- (a modular hardware controller (Palette Expert Kit; Palette; Canada)) and software (MAX 7; Cycling'74, USA) was modified and programmed by the author to suit the tinnitus matching needs of the studies.

1.3.2 Future Directions and Open Questions

In contrast to the part on neuroanatomy in this thesis, where the research questions and designs impose themselves in a pervasive manner almost automatically (i.e., also building 'on the shoulders of giants'), the task to sort in the novel approach of acoustic stimulation in existing literature, methodologies, and treatments is far more trickier. This is mostly

reflected by the approach sitting 'on the fence' between audiological assessment tools like RI tests (e.g., (Roberts et al., 2006, 2008)) and advanced partly already commercialized auditory therapies for tinnitus via reversal of maladaptive central plastivity (e.g., (Pantev et al., 2012; Tass et al., 2012)) or masking/retraining in the framework of tinnitus management (e.g., (Hoare et al., 2011; Henry et al., 2005a,b). Basically, the largest differences are to be found in presentation length where classical RI testing is applying sounds only for some few seconds whereas the auditory therapies focus on effects after several weeks of daily sound exposure for several hours. The novel approach presented here positions itself deliberately to time domains of minutes, in order to have both a better grip on prolonged RI and get a feel how this stimuli class may perform in the long run by also checking tolerability and possible unwanted side effects. Certainly, with this line of research progressing, the application of this stimuli class, adapted to longer stimulation in a daily life study design might be just around the corner.

Given these circumstances, there is only one more or less directly comparable study published so far besides the genuine studies presented in this thesis (Reavis et al., 2012). Notably, in the first part of the discussion in this study the considerations about the position of the novel paradigm between short- and long-term acoustic stimulation are comprehensively discussed similar to the considerations in the last paragraph of this thesis. However, several aspects of the distant relatives are also applicable to current experiments using the novel stimuli class of AM and FM sounds with different modulation rates. First of all, as observed in the study by Reavis et al. (2012), AM and FM sounds with carrier frequencies in the range of tinnitus does both induce better online (i.e., during stimulus presentation) masking as well as better RI after stimulus offset compared to unmodulated pure tone pendants and noise. Secondly, as reasoned in Reavis et al. (2012) and also theorized in the two studies (2 and 3) presented in this thesis with a different modulation rate, the observed on- and offline temporary tinnitus suppression may be mediated by a normalization of central aberrant neurophysiological activity. Reavis et al. (2012) wrote in their final remarks of the discussion that "Our result is most consistent with the tinnitus mechanism based on hyperactive neural activities in terms of increased spontaneous rate and increased within- and between-fiber synchrony throughout the entire central auditory pathway. Compared with pure tones and noises that mostly produce onset and offset auditory cortical activity, the present modulated stimuli produce robust

and sustained acoustically driven activity ... that may help restructure cortical firing patterns away from those that generate tinnitus. Moreover, the 40-Hz amplitude modulation should generate a strong a 40-Hz auditory steady-state response, enhancing the gamma rhythm to potentially disrupt thalamocortical dysrthythmia and drawing attention away from tinnitus". Therefore, a similar reasoning is therefore behind the approach of the stimuli class presented in studies 2 and 3 of this thesis with the difference that a modulation rate of 10 Hz is favored. Unlike in the former study, it is theorized that a modulation rate in the EEG alpha band (here: 10 Hz representing the middle frequency of the 8-12 Hz alpha band) may normalize deficient alpha activity in TI (Weisz et al., 2005, 2007; Schlee et al., 2014). Furthermore, it is speculated (in the absence of data) that slower modulations rates (here: 10 Hz >40 Hz) are more tolerable for application in auditory therapy for tinnitus, which may be elicited by basal ratings of valence and arousal (Bradley and Lang, 1994).

To sum up, both modulation rates and carrier frequencies have to be carefully studied to probe their specific individual as well as their joint effects on short-term tinnitus suppression. Beyond that, given the heterogeneity in tinnitus and individual responses or preferences, both the modulation rate and the carrier frequencies, and possibly the presentation loudness, may need individual adjustment for optimal efficacy in future therapeutic interventions with this stimuli class.

At this point in this new avenue of research in acoustic stimulation for tinnitus relief, several open questions have to be answered:

First, it is of central interest if tinnitus suppression is optimal with carrier frequencies at or around the (matched) tinnitus frequency (Reavis et al., 2012), or other carrier frequencies (or even sounds like noise, music etc.), might produce similar effects.

Second, the question arises if the manipulation of modulation rates produces differential tinnitus suppression. Moreover, it would also be interesting to study how and where the influence of the various modulation rates within the auditory pathway and the brain (e.g., on a cortical level measured with MEEG methods, or subcortically in animal models).

Third, feasibility, tolerability, and safety are features to be tested with respect to envisioned longitudinal studies. Therefore careful evaluation of pilot data and feedback from participants may be needed and, later on, subjective ratings for tolerability should

be assessed.

Fourth and related to that, sound or stimulation levels have to be manipulated to probe optimal efficacy of the stimuli. There are different approaches of sound levels while it is certainly comprehensible that a stimulation level at, slightly below, or above the tinnitus level may be more appropriate for long-term use than sound levels 65 dB SL up to 95 dB SPL (sounds were only presented for 30 seconds, though, (Roberts et al., 2008)).

Ultimately, due to the concurrent harsh and monotonic sonic image of these sounds (which are reduced to their core features due to the proof of principle phase of this stimuli class), aesthetically appealing forms of application have to be conceptualized, designed and tested for possible interventional use.

1.4 Aims and Significance

To conclude the introductory chapter, open questions of interest are highlighted, the aims of the three studies of this thesis are summarized, and finally results, significance, and novelty aspects of the studies are sketched out. Notably, every study was specifically designed to address open questions in tinnitus research as laid out comprehensively in the introduction and they are considered as perforce pieces towards better understanding of the tinnitus symptom.

Open questions for Study 1: Are there alterations in auditory cortex related to tinnitus which can be detected using SBM and correlational analyses within a large sample of TI? Do these putative alterations exert differential patterns of CT, CSA, or CV and can these patterns extend former findings of reduced cortical gray matter in auditory cortex? Is there a relationship between key tinnitus parameters like distress or duration and cortical morphology? Do these relationships inform us about neuroplasticity or predisposition of tinnitus with alterations in CT or CSA, respectively?

Study 1 (chapter 2.1, Meyer, Neff, Liem, Kleinjung, Weidt, Langguth, & Schecklmann, 2016) therefore re-analyzed a large sample of 256 TI using SBM (FreeSurfer) and GLM statistics to first try to replicate the former VBM finding and then perform analyses to extend former results alongside the open questions. Study results confirmed the bilateral decrease of gray matter in replicating the correlation with tinnitus distress. Notably, former results were extended with novelty as the negative correlation could be reduced to CSA, which points to a predispositional factor of tinnitus. Furthermore, this was the first independent study which could contribute neuroanatomical data to the proposed model of frontostriatal gating and deficient noise canceling in tinnitus. Finally, the pattern emerged that tinnitus distress is favorably related to reductions in CSA while tinnitus duration, a marker of chronification, was related to bi-directional alterations of CT in auditory and limbic structures.

Open questions for Study 2: Are amplitude modulated (10 Hz) sound exerting better short-term tinnitus suppression than their unmodulated pendants? Is this acoustic stimulation approach generally feasible and safe?

Study 2 (chapter 2.2, Neff, Michels, Meyer, Schecklmann, Langguth, & Schlee, 2017) therefore aimed at probing a wide array of common acoustic stimuli with or without am-

plitude modulation at a 10 Hz rate in an exploratory manner. Furthermore, feasibility and safety of the approach were assessed. Results indicated that amplitude modulated stimuli were partly exhibiting better short-term tinnitus suppression than their unmodulated pendants. While not all contrasts reached statistical significance, no stimulus of this novel stimulus class performed worse than the unmodulated class. Feasibility and safety could be demonstrated by good tolerability of the sounds and no adverse events or sustained increase in tinnitus loudness.

Open questions for Study 3: Do amplitude modulated sounds with different modulation rates exert differential short-term tinnitus suppression than their unmodulated pendants? Is there a difference in tinnitus suppression when the stimulation level is manipulated? How is the tolerability for the different stimuli elicited by valence and arousal ratings?

Study 3 (chapter 2.3, Neff, Michels, Meyer, Schecklmann, Langguth, & Schlee, submitted) therefore tested pure tones matched to the tinnitus frequency, which proved as most efficient in tinnitus suppression in study 2, both manipulated in modulation rates (i.e., 10 and 40 Hz) and stimulation level (i.e., 60 dB SL and 6 dB above MML). Furthermore, tolerability of the sounds was assessed with valence and arousal ratings. Results mostly fulfilled the hypotheses by confirming that both modulation rates but especially 10 Hz showed better tinnitus suppression at both stimulation level regimes. This finding is insofar novel, that this is the first study showing that matched amplitude modulated sounds in the tinnitus frequency elicit better tinnitus suppression than pure tones, which are sometimes used as maskers. Beyond that, arousal and especially valence ratings were significantly better for the (10 Hz) amplitude modulated sounds.

2 Manuscripts

2.1 Differential tinnitus-related neuroplastic alterations of cortical thickness and surface area

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A similar version of this manuscript has been published in Hearing Research 342 (2016) 1-12, DOI :10.1016/j.heares.2016.08.016

Abstract

Structural neuroimaging techniques have been used to identify cortical and subcortical regions constituting the neuroarchitecture of tinnitus. One recent investigation used voxel-based morphometry (VBM) to analyze a sample of tinnitus patients (TI, n=257) (Schecklmann et al., 2013). A negative relationship between individual distress and cortical volume (CV) in bilateral auditory regions was observed. However, CV has meanwhile been identified as a neuroanatomical measurement that confounds genetically distinct neuroanatomical traits, namely cortical thickness (CT) and cortical surface area (CSA). We performed a re-analysis of the identical sample using the automated FreeSurfer surface-based morphometry (SBM) approach (Fischl, 2012). First, we replicated the negative correlation between tinnitus distress and bilateral supratemporal gray matter volume. Second, we observed a negative correlation for CSA in the left peri-auditory cortex and anterior insula. Furthermore, we noted a positive correlation between tinnitus duration and CT in the left peri-auditory cortex as well as a negative correlation in the subcallosal anterior cingulate, a region collated to the serotonergic circuit and germane to inhibitory functions.

In short, the results elucidate differential neuroanatomical alterations of CSA and CT for the two independent tinnitus-related psychological traits distress and duration. Beyond this, the study provides further evidence for the distinction and specific susceptibility of CSA and CT within the context of neuroplasticity of the human brain.

Introduction

Tinnitus can be conceived as an auditory phantom perception of transient or permanent sound, noise, or ringing without any corresponding external sound source (Eggermont and Roberts, 2004). In Western industrialized countries with steadily aging populations and enhanced exposure to environmental noise, the number of individuals who suffer from tinnitus is substantial (Gallus et al., 2015). According to recent estimations, approximately 50 million people in the US and 70 million people in the European Union are affected by tinnitus (Cederroth et al., 2013). Although previous research and treatment focused on the inner ear, it has since been widely accepted that tinnitus should not be considered as a sole dysfunction of the ear, even though tinnitus is usually preceded by and associated with substantial to minor or even hidden peripheral hearing loss (Knipper et al., 2013; Roberts et al., 2012). Instead, it has widely been agreed that tinnitus emanates from a perplexing network that includes the inner ear, the auditory pathway, and non-auditory brain areas (De Ridder et al., 2011a, 2014; Elgoyhen et al., 2012; Jastreboff, 1990; Rauschecker et al., 2010).

In particular, as there is presently no effective medical or psychological therapy available to cure tinnitus, it is of the utmost importance to better understand the sensory and cognitive mechanisms that directly or indirectly may result in alterations of cortical architecture. Careful research of the circumstances and conditions under which these changes occur may help to answer a number of pertinent questions. One important issue relates to the heterogeneity of the TI population which may be subdivided into a number of various tinnitus subtypes (Knipper et al., 2013; Schecklmann et al., 2012). A relevant question in this context pertains to the role that one cardinal feature, namely emotional distress,[1] plays in tinnitus subtyping (Meyer et al., 2014b; De Ridder et al., 2011b; Golm et al., 2013; Vanneste et al., 2010). Recent research has shown that there is a high variability among people with tinnitus in the degree to which they are emotionally affected by the chronic noise (Milerova et al., 2013). While some TI learn to ignore and to cope with the disturbing noise, others begin to develop symptoms of mental, psychological and emotional disorders. In the absence of any reasonable and appropriate coping strategies, these persons consider the permanent sound to be extremely detrimental (Vanneste et al., 2014).

[1] Emotional distress in TI is measured by a standard self-report questionnaire, the Tinnitus Questionnaire (TQ) (Goebel and Hiller, 1998; Hallam, 1996)

It has been suggested that the neural thalamo-cortical circuit that maintains the phantom sound connects increasingly with attentional circuits, and that this neural loop, being further accelerated by aversive emotional attributions, is continuously updated, eventually becoming chronically established (De Ridder et al., 2011a; De Ridder et al., 2015). Thus, chronic subjective tinnitus could be considered a disorder that results from the maladaption of several overlapping brain systems that bind together in a 'vicious cycle' guided by principles of neural learning mechanisms. A recent paper takes an alternate stance by describing parallel networks that may differentially contribute to the experience of auditory and emotive-related tinnitus symptoms (De Ridder et al., 2014). According to this model, deprived sensory inflow following damage in the peripheral hearing system impedes inhibitory circuits in the central peri-auditory nervous system. Whether fronto-cingulate-insular circuits are able to tone down the excitation of the central auditory functioning may vary considerably between individuals. In highly distressed TI various prefrontal, insular, and anterior and posterior cingulate regions may be conceived as being key nodes in this network that maintains the tinnitus experience (Schlee et al., 2009).

With the advent of neuroimaging techniques a considerable number of functional brain scan studies have been performed (for reviews, see (Adjamian et al., 2009; Lanting et al., 2009)). The number of studies that scrutinized the neuroanatomical changes in the brain structure of TI is smaller, but for the time being, the results are notably inconsistent and heterogeneous (Aldhafeeri et al., 2012; Boyen et al., 2013; Husain et al., 2011; Landgrebe et al., 2009; Leaver et al., 2011; Melcher et al., 2013; Mühlau et al., 2006) (for a review, see (Adjamian et al., 2014)). The range of brain regions that appear to undergo structural changes either as a function or as a catalyst of chronic noise perception includes the supratemporal, the lateral (pre-)frontal cortex, medial frontal, cingulate, temporal, subcallosal and parietal cortex as well as a number of subcortical nuclei. In particular, bilateral peri-auditory regions in the superior temporal lobe (Aldhafeeri et al., 2012; Boyen et al., 2013; Husain et al., 2011) and subcortical areas that are considered as part of the (anatomically ill-defined) limbic system (Landgrebe et al., 2009; Leaver et al., 2012) often show anatomical changes. However, to date, neither the direction of tinnitus-related changes (increase or decrease of neuroanatomical gray matter (GM)) is clear, nor is it clarified whether the changes in the neural architecture of distinct regions occur independently or are related. The substantial discrepancy between studies is confusing and makes the

interpretation of the (sometimes conflicting) data problematic. The methodological impediments that may complicate the comparability of studies on structural neuroplasticity in tinnitus are small sample sizes, comorbid psychological problems, differences in TI's age of onset and duration, that is the interval between tinnitus onset and the time point of data acquisition. Furthermore, evidence for confined "structural abnormalities specifically related to tinnitus is sparse" (Adjamian et al., 2014, p. 119), and this fact makes it difficult to test specific anatomical hypotheses. The results obtained from studies that used the standard VBM approach (Boyen et al., 2013; Husain et al., 2011; Landgrebe et al., 2009; Leaver et al., 2011; Mühlau et al., 2006; Schecklmann et al., 2013; Vanneste et al., 2015) are difficult to reconcile due to their heterogeneity. Thus the question arises whether the inconsistency of available results may be related to methodological limitations of VBM measurements (Adjamian et al., 2014). Certainly, VBM results are not straightforwardly comparable to the results of studies that applied a more innovative approach, namely 'surface-based morphometry' (SBM) in their investigations into the structural signature of tinnitus (Leaver et al., 2012).

As Panizzon and colleagues (Panizzon et al., 2009) argued, cortical volume (CV) as measured by the VBM approach is approximately the product of cortical surface area (CSA) and cortical thickness (CT). Therefore, if these variables run in opposite directions, CV measurements could be confounded and tinnitus-related alterations might be obscured. Since that publication a number of investigations empirically disentangled CSA measurements from the quantification of thickness (Chiarello et al., 2013; Greve et al., 2013; Hogstrom et al., 2013; Koelkebeck et al., 2014; Koolschijn and Crone, 2013; Lyttelton et al., 2009; Meyer et al., 2014a; Raznahan et al., 2011; Vuoksimaa et al., 2015; Wierenga et al., 2014). Sustaining this view, Bermudez and colleagues demonstrated that the perisylvian brain anatomy of musicians varies as a function of the particular measurement methodology being used (VBM vs. measurement of CT by SBM) (Bermudez et al., 2009). These studies provide strong evidence that cortical thickness and surface may differ in their relationship to behavioral traits and hence may confound CV measurements.

With respect to all this heterogeneity in both the applied measurement techniques and the experimental operationalizations, it is advisable to proceed in small steps. Following suggestions by other scholars (Adjamian et al., 2014; Elgoyhen et al., 2015), our approach favors the replication of findings across independent research groups, as well as

structural imaging methods (i.e., VBM and SBM) to increase both confidence and credibility of results (Leaver et al., 2012). Hence, we decided to replicate a large sample of recently published data that was intended to measure tinnitus-related structural changes of the human cortex (Schecklmann et al., 2013). In this study, VBM was used to elucidate tinnitus-related structural alterations in a sample of only TI. According to the authors, tinnitus-related distress correlated negatively with CV in peri-auditory areas, namely Heschl's gyrus and the bilateral insula. This relationship remained stable even after correcting for age, sex, hearing loss, and further covariates.

We consider this set of data as the most suitable for a replication for several reasons. First, the sample size (n=257) is expected to elicit high statistical power and a reliable cross-sectional representation of various tinnitus-related psychopathological and neuroanatomical aspects. Second, the sample is clinically well-characterized. Third, we think that tinnitus-related distress may prove to be a revealing trait as the investigation of its variance in the TI population in correspondence with other behavioral, neurophysiological, structural and psychological parameters may form an ideal platform for the study of the perplexing pathophysiology of tinnitus beyond the otological and audiological aspects. Unlike Schecklmann and colleagues (Schecklmann et al., 2013) we applied an established SBM approach, namely the Freesurfer software suite (Dale et al., 1999; Fischl et al., 1999a), which allows for the disentanglement of CT from CSA, thus enabling a more differentiated and detailed picture of the relationship between tinnitus-induced anatomical changes and the tinnitus-related psychometric traits of distress and duration. By using this approach, we are in keeping with the suggestion of Scheckelmann and colleagues (Schecklmann et al., 2013, p. 1068) who recommended "the use of rather individualized strategies such as Freesurfer".

In sum, the present study is primarily intended to be a replication of the work by Schecklmann and co-scholars (Schecklmann et al., 2013) who analyzed a large and homogeneous sample of TI by means of the standardized VBM approach. For the current analyses we used the identical sample of individuals who suffer from chronic subjective tinnitus but vary in their individually experienced emotional distress as quantified by the Tinnitus Questionnaire (TQ) (Goebel and Hiller, 1998). Akin to Schecklmann and colleagues, we expect to find a negative relationship between tinnitus-related distress and CV, at least in bilateral peri-auditory regions. Furthermore, we hypothesize that in our analysis the same

pattern will be observed for CSA as this neuroanatomical trait has been found to correlate strongly with CV (Greve et al., 2013; Meyer et al., 2014a). Regarding tinnitus duration, we are not able to provide well-based hypotheses; however, we expect to find alterations of thickness, which is believed to be a reliable marker of pathology-related as well as lifespan neuroplasticity. At the very least, we postulate that an analysis of CV, CSA, and CT will yield results showing differences in plastic changes when distress is compared to duration, as we consider these two measures to be unrelated aspects of chronic subjective tinnitus (Meyer et al., 2014b; Schecklmann et al., 2013).

Methods

Participants

We re-analyzed structural MRI data of 257 participants (73 female) who took part in the study of Schecklamm and co-scholars (Schecklmann et al., 2013). The mean age was 50 ± 12 (range 16-77). All participants had a diagnosis of subjective tinnitus. The participants underwent comprehensive otological and audiological tests. Hearing function was defined as the mean threshold, and averaged over the frequencies 0.125, 0.250, 0.500, 1, 2, 3, 4, 6, and 8 kHz (left ear: 18 ± 15 (0-114) dB HL; right ear: 17 ± 13 (0-89) dB HL). The slope of the audiogram was taken into account by computing the hearing level difference of the pair of neighboring frequencies with the highest hearing level difference (left ear: 20 ± 12 (0-50) dB/octave; right ear: 19 ± 12 (0-70) dB/octave) (Schecklmann et al., 2013).

We excluded patients who displayed hints of Ménière's disease, namely vertigo in combination with tinnitus, or who showed signs of objective, pulsatile tinnitus, that is a sound generated by a physical source, such as a vascular malformation. None of the patients reported any history of severe illness or exhibited contraindications for MRI scans (e.g., cardiac pacemakers or other implanted electronic devices, claustrophobia, etc.). All MRI scans were visually inspected. If there were artifacts or signs of brain malformation, patients were not included in the study. All participants gave their written, informed consent after a comprehensive introduction covering the experimental procedures. The study was approved by the Ethics Committee at the University of Regensburg. All procedures in-

volved were conducted in accordance with the Declaration of Helsinki prior to the last revision in 2013 as the original data had been collected between 2004 and 2009.

Questionnaire

A German adaptation of the Tinnitus Questionnaire (TQ) was applied to assess tinnitus-related information (Goebel and Hiller, 1998). TQ is a widely established instrument to assess tinnitus-related distress. It comprises 52 statements, which are judged on a three-point Likert scale ('true', 'partially true', 'not true'). The TQ has a factor structure that reveals the total score for tinnitus distress and severity, as well as six subscores ('Cognitive Distress', 'Emotional Distress', 'Intrusiveness', 'Auditory Perceptual Difficulties', 'Sleep Disturbances', and 'Somatic Complaints').

MRI data acquisition and analysis

MRI data acquisition

Magnetic resonance imaging (MRI) scans were acquired using a Siemens Sonata scanner at 1.5T and a standard 8-channel birdcage head coil. A 3 dimensional structural MRI was acquired for each participant using a T1-weighted magnetization rapid gradient echo sequence (time of repetition 1880 ms; echo time 3.42 ms; time to inversion 1100 ms; flip angle $15°$; matrix size 256x256), which yielded 76 sagittal slices with a defined voxel size of 1x1x1 mm. The scanner was upgraded twice within the measurement interval of five years (2004-2009). Consequently, an analysis of covariance was done to statistically control for this potential confound.

Surface-based morphometry

The reconstructions of cortical surface and volumetric segmentation were performed with the concurrent FreeSurfer image analysis suite (version 5.3.0). This software is documented online and freely available for download *(http://freesurfer.net)*. The technical details of these procedures are described in prior publications (Dale et al., 1999; Fischl et al., 1999a,b, 2001, 2002, 2004a; Segonne et al., 2004). In short, the FreeSurfer pipeline generates models of the individual cortical surface with sub-voxel/-millimeter precision, yielding measures of CT, CSA and CV at each vertex of the surface. The fully automated

procedure involves preprocessing of the subject's image data, segmentation of the cortical white and gray matter (GM/WM), tessellation of the GM/WM junction, inflation of the folded surface tessellation patterns, and automatic correction of topological defects. Notably, the procedures for measuring CT have been validated against both histological analysis (Rosas et al., 2002) and manual measurements (Cardinale et al., 2014; Kuperberg et al., 2003; Salat et al., 2004), and have been shown to be reliable (Liem et al., 2015).
For the statistical analysis, each participant's reconstructed brain was morphed to an average spherical surface and smoothed using a FWHM kernel of 10 mm as applied in previous work (Leaver et al., 2012).
CT is defined by the shortest distance between the gray/white matter border and pial surfaces, while CSA is the mean area of the triangular region at the respective surface data point (vertex). Approximately, CV is the arithmetic product of CSA by CT. Indexes of gyrification at each vertex, as an added explaining factor for differences in volume, were also taken into consideration.
In addition, the cerebral cortex was parcellated into units based on gyral and sulcal structure, thus enabling the respective ROI statistics (Desikan et al., 2006; Destrieux et al., 2010; Fischl et al., 2004b). All subjects were analyzed on a Sun Microsystems HPC cluster running Linux SLES11 SP1. A visual inspection was performed randomly on selected subjects. At no time were the results manually edited.

Statistical analysis

To ensure an optimal comparability to the previous VBM analyses, whole-brain analyses using the built-in GLM of FreeSurfer were computed. Akin to the study of Schecklmann and co-workers (Schecklmann et al., 2013) the following steps of data analysis were performed: To start, we used tinnitus distress as measured by TQ as a single regressor in a principal model that does not specifically consider possible confounds. This model will be termed 'model without covariates' (MOC) throughout the remainder of this manuscript. To follow, a model with covariates (MWC) investigating the role of the cardinal nuisance factors, namely age, sex, hearing level (Adjamian et al., 2014), and further factors such as tinnitus duration, laterality, audiometric slope and scanner upgrade (Schecklmann et al., 2013) was calculated.

This particular strategy was carefully deployed and evaluated in accordance with the previous work of Schecklmann and colleagues (Schecklmann et al., 2013, p. 1064), as covariates can engender or annihilate statistical effects (Miller and Chapman, 2001). For the statistical analysis of both models, we used a significance threshold of 0.001 at vertex level analogously to the previous VBM analysis and a recent study by Vanneste and co-authors (Vanneste et al., 2015). Both models were corrected for multiple comparisons for each hemisphere independently (FDR, $p < 0.05$ and Monte Carlo Null-Z simulation with an initial vertex-threshold of $p = 0.001$ and a cluster threshold of $p = 0.05$). We also report standard statistics without any correction with a threshold of 0.001 at vertex level.
Further to this, we applied a multiple regression analysis (MWC) in SPSS 21 (SPSS Inc., Chicago, IL) in order to conduct anatomical ROI analyses of the bilateral primary auditory cortices (AC) using the cortical parcellations of FreeSurfer (Desikan et al., 2006). The AC ROI was defined as being the bilateral transverse temporal gyrus (Heschl's gyrus) by the atlas of (Desikan et al., 2006), which serves as the reference for the nomenclature of brain regions presented in Tables 1-6. Bonferroni adjustment was applied to control for multiple comparisons on the selected ROIs.
Finally, we used the volumetric estimates of subcortical structures independent of the cortical surface reconstruction stream within the FreeSurfer pipeline in an additional exploratory ROI analysis. ROIs related to the auditory system or tinnitus (models) were chosen out of the available subcortical structures, which resulted in a set including bilateral amygdala (Rauschecker et al., 2010), hippocampus (Landgrebe et al., 2009), nucleus accumbens (Rauschecker et al., 2010), and thalamus (Mühlau et al., 2006). The uncorrected results and Bonferroni-adjusted p-values are indicated in Table 7.

Results

Comprehensive demographic and tinnitus characteristics of the TI have been indicated by Schecklmann and collaborators (Schecklmann et al., 2013). The cortical reconstruction pipeline failed for one participant, leaving n=256 cases for further analysis. All other results of our SBM analysis are plotted in Figures 1-5 and Tables 1-7, and are described in the following paragraphs. Regarding the psychometric results, it is noteworthy that tinnitus distress as measured by the TQ total score and tinnitus duration do not correlate ($rho = 0.059, p = 0.345$).

The description of our anatomical results is organized according to the following structure: We start by delineating the findings for distress, and then follow these with the results for duration for each of the cortical parameters, namely volume, surface area, and thickness, respectively (Tables 1-6).

The analysis of the correlation between CV and distress in TI was intended to replicate the negative relationship observed by Schecklmann and co-authors (Schecklmann et al., 2013). Tables 1 and 2 list a comprehensive overview of the results for the correlations between CV and tinnitus distress. Notably, the analysis according to the MOC yielded a similarly weak effect (left AC $r = -0.284$, $p < 0.001$, right AC $r = -0.25$, $p < 0.001$) for the resulting clusters with the volume inversely related to tinnitus distress in both the left and the right auditory cortex situated on the supratemporal plane (cf. Table 3 of (Schecklmann et al., 2013)). The comparable peak vertices are indicated by the green crosshairs (Figure 1).

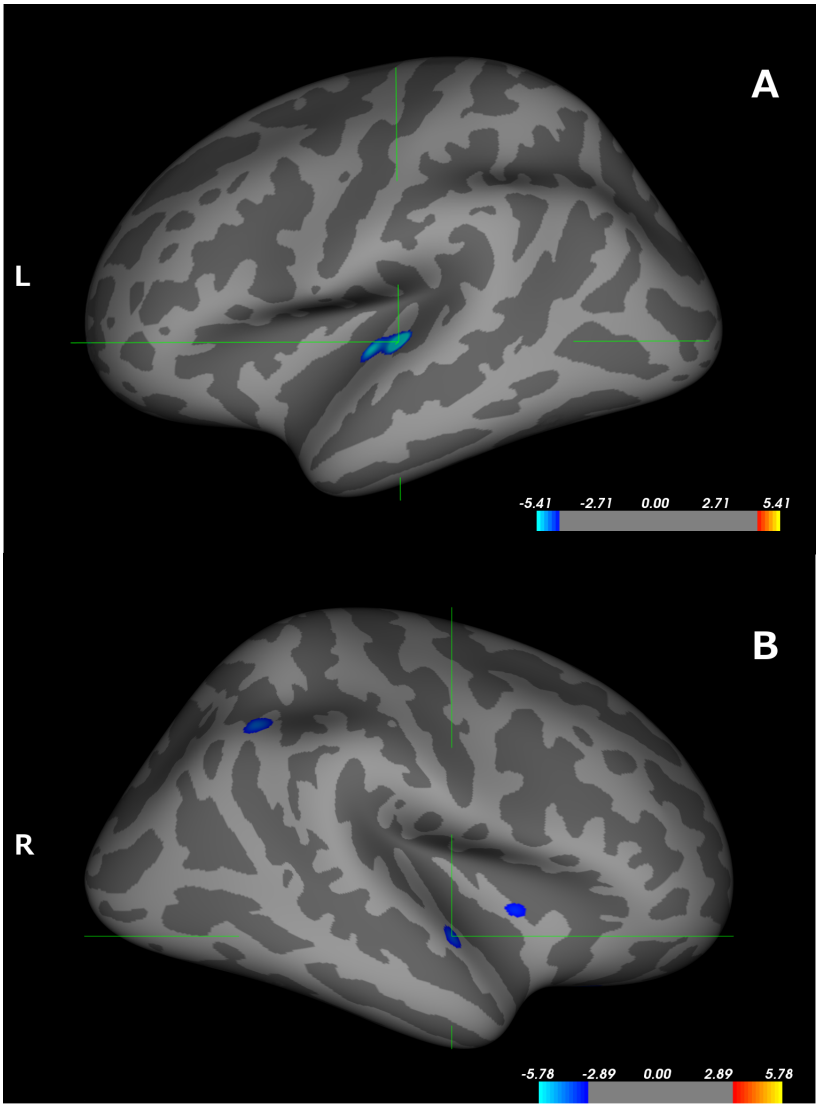

Figure 2.1: Negative correlations of tinnitus distress with CV in the left hemisphere (A) and the right hemisphere (B) of MOC. Green cross hairs indicate peak vertices (A: MNI -51 -21 4, B: MNI 59 -1 -4) comparable to peak voxels of VBM analysis (cf. figure 1 of (Schecklmann et al., 2013)).

				MNI				
Annotation	Max	NVts	Size (mm2)	X	Y	Z	NVts FDR	CWP MCZ
LH								
transverse temporal	-5.42	888	408.44	-51	-21	4	74	0.0007
inferior temporal	-4.27	111	63.52	-44	-50	-12		
rostral middle frontal	-4.11	234	173.4	-20	56	-2		0.0473
superior frontal	-3.67	195	102.13	-7	27	55		
rostral middle frontal	-3.32	81	68.4	-23	54	16		
superior frontal	-3.31	67	26.18	-7	52	36		
superior temporal	-3.30	28	23.63	-48	9	-25		
postcentral	-3.14	60	28.6	-32	-31	61		
RH								
lateral orbitofrontal	-5.69	656	281.21	16	21	-17	486	0.0059
inferior parietal	-4.58	378	171.66	45	-55	43	230	
superior temporal	-4.30	228	101.31	59	-1	-4	114	
parahippocampal	-4.11	122	49.09	37	-35	-15	56	
insula	-3.91	175	80.31	36	3	1	67	
cuneus	-3.72	151	120.41	5	-77	16	25	
transverse temporal	-3.40	52	20.11	43	-24	3		
rostral middle frontal	-3.27	33	20.17	37	51	9		
rostral middle frontal	-3.23	84	59.9	16	21	-17		
pars orbitalis	-3.20	32	26.06	45	-55	43		
lateral orbitofrontal	-3.13	14	8.44	59	-1	-4		
superior temporal	-3.08	17	6	37	-35	-15		

Table 2.1: Statistics of the negative correlations of cortical volume and tinnitus distress (MOC). Left hemisphere (LH): Max: -log10(p) at peak vertex (values > 3 correspond to $p < 0.001$), NVts: number of vertices above threshold ($p < 0.001$, uncorrected), NVts FDR: number of vertices above threshold ($p(FDR) < 0.000022$), CWP MCZ: cluster-wise p-value of Monte Carlo Null-z simulation (vertex-wise/initial $p = 0.001$). Right hemisphere (RH): $p(FDR) < 0.00029$.

				MNI		
Annotation	Max	NVts	Size (mm2)	X	Y	Z
LH						
inferior temporal	-4.45	113	124.04	-45	-50	-12
rostral middle frontal	-3.29	29	95.66	-20	55	-3
superior temporal	-3.07	7	70.18	-47	8	-26
RH						
insula	-5.34	232	169.49	39	1	1
inferior parietal	-3.48	137	315.75	44	-63	39
inferior parietal	-3.39	65	114.12	43	-66	25
inferior temporal	-3.31	28	69.94	55	-23	-24
inferior parietal	-3.15	36	20.11	46	-56	44

Table 2.2: Statistics of the negative correlations of cortical volume and tinnitus distress in both hemispheres (MWC). Max: -log10(p) at peak vertex (values > 3 correspond to $p < 0.001$), NVts: number of vertices above threshold ($p < 0.001$, uncorrected).

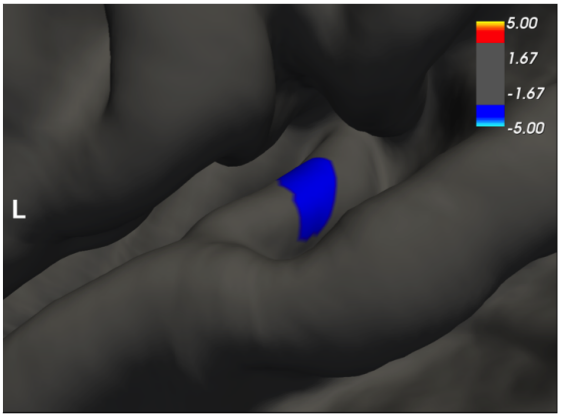

Figure 2.2: Negative correlation of tinnitus distress with CSA in the left AC (MOC. See tables 3 and 4 for more details.).

				MNI		
Annotation	Max	NVts	Size(mm2)	X	Y	Z
LH						
superior frontal	-3.80	201	100.06	-7	28	54
lateral occipital	-3.49	224	197.41	-20	-97	5
fusiform	-3.46	129	71.42	-41	-49	-17
transversetemporal	-3.30	107	41.61	-47	-25	8
RH						
superior temporal	-4.61	314	127.32	64	-37	16
lateral orbitofrontal	-3.05	12	8.27	15	46	-19

Table 2.3: Statistics of the negative correlations of cortical area and tinnitus distress in both hemispheres (MOC). LH: Left hemisphere, RH: Right hemisphere, Max: -log10(p) at peak vertex (values > 3 correspond to $p < 0.001$), NVts: number of vertices above threshold ($p < 0.001$, uncorrected).

				MNI		
Annotation	Max	NVts	Size (mm2)	X	Y	Z
LH						
fusiform	-3.56	79	38.31	-40	-46	-17
lateral occipital	-3.26	57	35.82	-14	-99	13
superior temporal	-3.12	30	14.78	-54	-26	1
lateral orbitofrontal	-3.09	14	4.98	-28	24	2
superior frontal	-3.05	7	4.07	-7	29	52
posterior cingulate	-3.04	8	2.64	-11	-2	40
RH						
lateral occipital	-3.30	86	74.52	30	-93	-1
superior temporal	-3.28	83	30.81	64	-38	17
inferiorparietal	-3.18	54	27.19	47	-62	35
fusiform	-3.16	44	21.92	43	-51	-12
medialorbitofrontal	-3.01	3	2.0	8	41	-11
insula	-3.01	2	1.0	36	4	1

Table 2.4: Statistics of the negative correlations of cortical surface area and tinnitus distress in both hemispheres (MWC). LH: Left hemisphere, RH: Right hemisphere, Max: -log10(p) at peak vertex (values > 3 correspond to $p < 0.001$), NVts: number of vertices above threshold ($p < 0.001$, uncorrected).

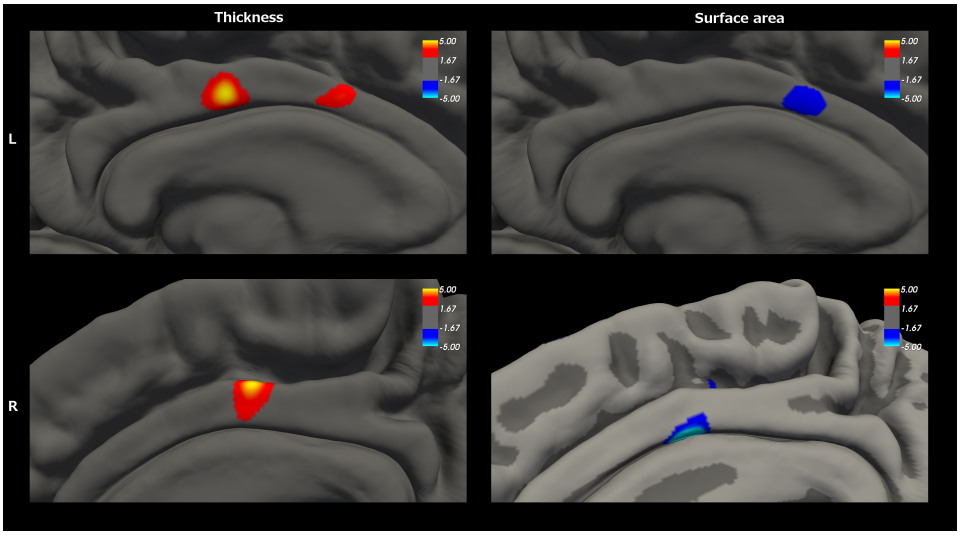

Figure 2.3: Correlations of tinnitus distress with CT (left panel) and CSA (right panel) in bilateral cingulate cortex (MWC, thresholded at p=0.005 for illustration. See tables 5 and 6 for more details.).

				MNI		
Annotation	Max	NVts	Size(mm2)	X	Y	Z
LH						
superior temporal	-4.29	163	75.47	-47	-11	-13
supramarginal	-4.24	107	43.58	-53	-32	37
lingual	-4.11	99	65.72	-12	-71	3
inferior parietal	-3.73	79	51.64	-39	-78	26
middle temporal	-3.73	121	73.72	-61	-45	-7
rostral middle frontal	-3.62	89	57.73	-23	42	32
caudal middle frontal	-3.29	36	17.8	-35	4	32
rostral middle frontal	-3.08	32	20.51	-33	49	7
RH						
medial orbitofrontal	-3.76	60	32.82	10	22	-18
lingual	-3.64	93	31.76	23	-51	0
superior temporal	-3.56	114	71.68	57	-6	-9
inferior parietal	-3.15	22	9.12	47	-54	45
superior frontal	-3.02	4	3.46	11	55	12

Table 2.5: Statistics of the negative correlations of cortical thickness and tinnitus distress in both hemispheres (MOC). LH: Left hemisphere, RH: Right hemisphere, Max: -log10(p) at peak vertex (values > 3 correspond to $p < 0.001$), NVts: number of vertices above threshold ($p < 0.001$, uncorrected).

				MNI		
Annotation	Max	NVts	Size (mm2)	X	Y	Z
LH						
posterior cingulate	3.10	16	5.49	-4	-15	35
superior temporal	-3.04	6	2.7	-47	-9	-13
RH						
posterior cingulate	3.00	1	0.45	6	-7	40

Table 2.6: Statistics of the positive and negative correlations of cortical thickness and tinnitus distress in both hemispheres (MWC). LH: Left hemisphere, RH: Right hemisphere, Max: -log10(p) at peak vertex (values > 3 correspond to $p < 0.001$), NVts: number of vertices above threshold ($p < 0.001$, uncorrected).

	distress			duration		
	r	p-value	p-bonf	r	p-value	p-bonf
Left Amy	-.052	.347	1	0.11	.047	0.376
Left HC	-.047	.403	1	.015	.783	1
Left Nacc	-.074	.159	1	-.028	.593	1
Left Tha	-.128	.009	.072	.063	.2	1
Right Amy	-.061	.279	1	-.009	.872	1
Right HC	-.033	.565	1	.022	.697	1
Right Nacc	-.001	.989	1	-.008	.87	1
Right Tha	-.021	.688	1	.066	.203	1

Table 2.7: ROI analysis of subcortical structures: Correlations of tinnitus distress and duration with subcortical volumes as elicited with multiple regression analysis (MWC). Amy=Amygdala, HC=Hippocampus, Nacc=Nucleus accumbens, Tha=Thalamus, p-bonf= bonferroni-adjusted p-value.

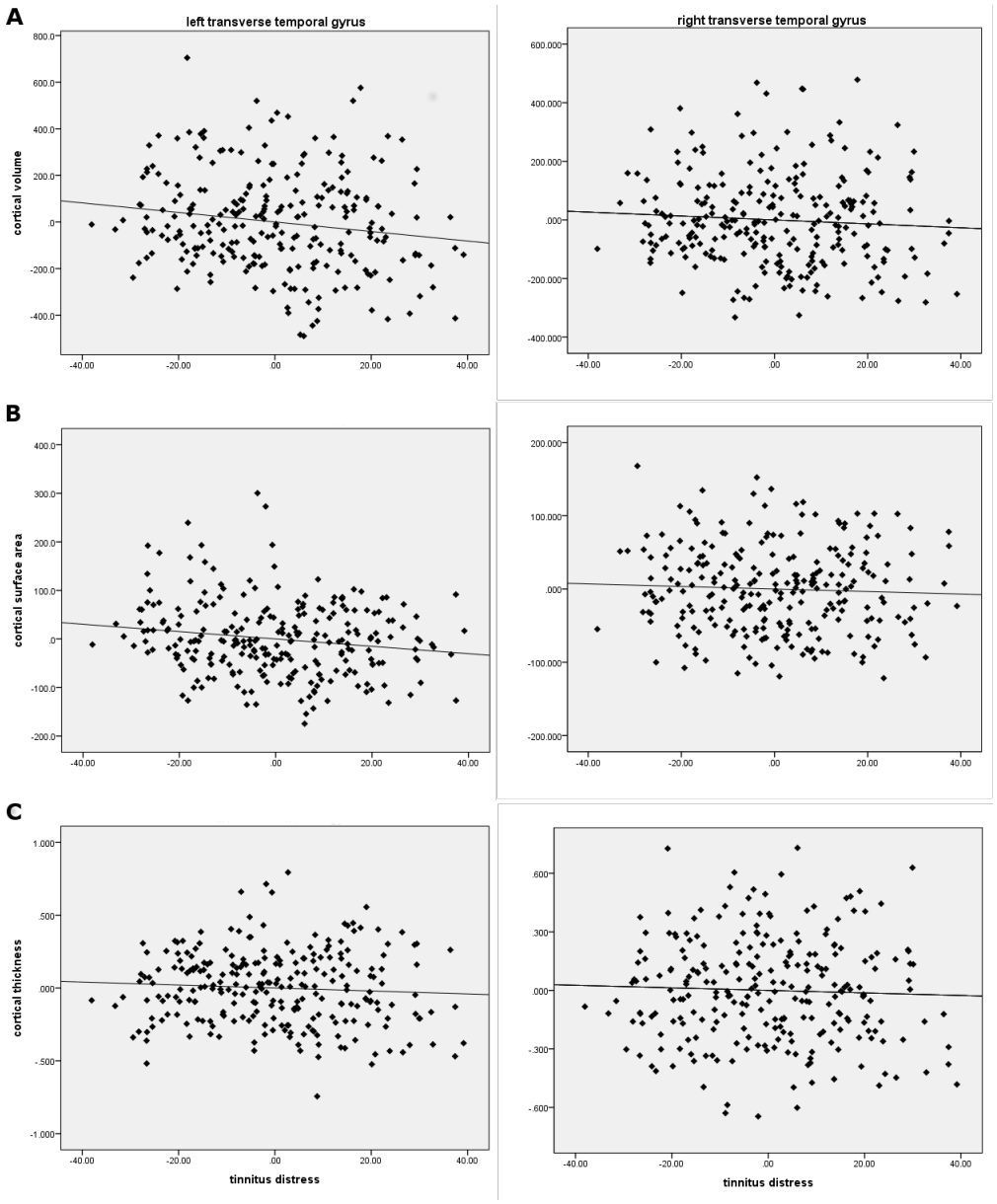

Figure 2.4: ROI analysis: Negative correlations of tinnitus distress with CV and CSA but not CT in the left transverse temporal gyrus as elicited by multiple regression (MWC). A: Partial regression plot of tinnitus distress vs. left transverse temporal gyrus cortical volume: r=-0.149, p=0.024* and right transverse temporal gyrus cortical volume: r=-0.066, p=0.544. B: Partial regression plot of tinnitus distress vs. left transverse temporal gyrus cortical surface area: r=-0.151, p=0.018* and right transverse temporal gyrus cortical surface area: r=-0.046, p=0.892. C: Partial regression plot of tinnitus distress vs. left transverse temporal gyrus cortical thickness: r=-0.063, p=0.608 and right transverse temporal gyrus cortical thickness: r=-0.039, p=1.054.

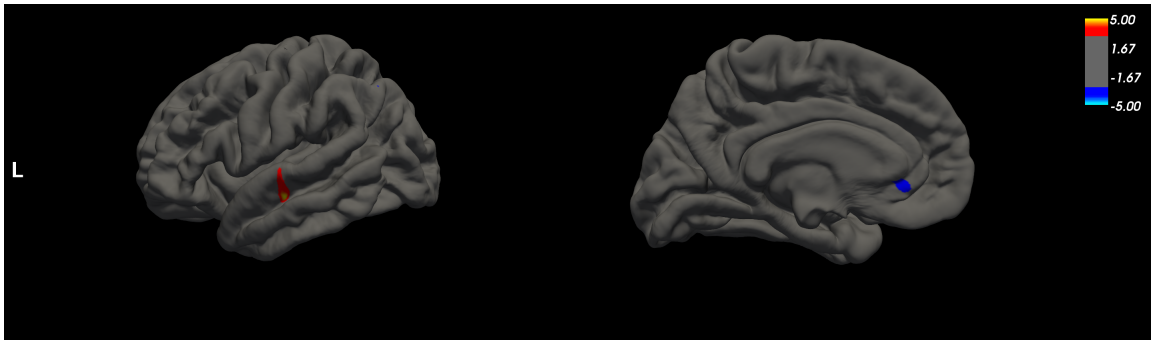

Figure 2.5: Correlations of tinnitus duration with CT in the left hemisphere (MWC). Left panel: positive correlation of tinnitus duration with CT in STS. Right panel: negative correlation of tinnitus duration with CT in sgACC.

When specifically addressing CSA the following pattern is remarkable (cf. Figure 2). We observed a negative correlation in the left auditory cortex ($-log10(p) = -3.30$, $r = -0.229$, $p < 0.001$, number of vertices (NOV) = 107, MNI = -47 -25 8) whereas the analysis of CT did not yield any significant results in this region. According to the whole-brain analyses, the effect was only significant in the left auditory area.

Tables 3 and 4 show that other regions also reveal a significant negative relationship between CSA and distress in distinct bilateral areas of the cortex, these being a lateral occipital region, an inferior parietal region, a superior frontal region, the unilateral right medial plane (cuneus), as well as the medial plane with anterior/posterior cingulate cortex.

Regarding CT, we found positive correlations for the medial plane, namely for the left and right medially situated posterior cingulate (Figure 3, Table 6). Apart from the correlations in PCC, thickness did not yield any significant positive correlations at the thresholded p-value (0.001), and only one negative correlation in the anterior superior temporal region (Table 6).

In line with our predictions, the additional multiple regression analysis on the anatomical ROI mean values controlling for all confounds (MWC) showed a significant negative correlation between tinnitus distress and neuroanatomical traits in the left core auditory ROI for both CV ($r = -0.149$, $p = 0.024$ (Bonferroni-adjusted) and CSA ($r = -0.151$, $p = 0.018$), whereas no significant effect was found for CT ($r = -0.063$, $p = 0.608$) (Figure 4). In the right hemisphere, the pattern of results for the MWC model differs slightly, as we found no significant negative relationship for tinnitus distress and neuroanatomical parameters for all three traits, namely for CV ($r = -0.066$, $p = 0.544$), CSA ($r = -0.046$, $p = 0.446$), and CT ($r = -0.039$, $p = 1.054$). No significant correlations between tinnitus distress and gyrification indexes were found ($p < 0.001$).

For tinnitus duration we observed significant effects in two regions, and only for thickness (Figure 5). We observed a negative correlation between duration and CT in the subcallosal anterior cingulate adjacent to the ventral striatum ($-log10(p) = -3.75$, $r = -0.251$, $p < 0.001$, NOV = 103, MNI -6 30 -5) and a positive relationship between duration and CT in the right anterior superior temporal lobe (covering the lateral convexity of the superior temporal gyrus (STG) and the dorsal banks of the superior temporal sulcus (STS)) ($-log10(p) = 4.25$, $r = 0.238$, $p < 0.0001$, NOV = 263, MNI = -51 -14 -11). It is noteworthy that no significant correlations of CSA and CV with duration were found (MWC,

$p < 0.001$). It should be highlighted that tinnitus distress and duration were mutually controlled for by including them as covariates in the respective GLM models of the FS whole-brain analysis as well as the multiple regression analysis. The duration-CT findings reported in this manuscript are clearly distinct from the patterns of the distress-CSA correlations. As a final point, no significant correlations between tinnitus duration and gyrification indexes were found ($p < 0.001$).

For the sake of completeness, Table 7 indicates the results of the multiple regression analysis on the subcortical ROI volumes with tinnitus distress and duration. Notably, the volume of the left thalamus is weakly negatively correlated with tinnitus distress ($r = -0.128$, $p = 0.009$, uncorrected) whereas the left amygdala is positively correlated with tinnitus duration ($r = 0.110$, $p = 0.047$, uncorrected).

In sum, our SBM analysis revealed several specific relationships between tinnitus related behavioral indications (distress, duration) and neuroanatomical traits (CV, CSA, CT). More important, we replicated the negative relationship between individual distress and cortical volume in bilateral auditory fields that had formerly been revealed by VBM analysis (Schecklmann et al., 2013). Our analysis, however, also provides an additional value; regarding CSA and CT (which were not analyzed by the former VBM study) we observe a differential pattern of negative and positive correlations with distress and duration in the same sample of TI.

Discussion

In this section we begin by contrasting the pros and cons of the evaluated procedures for measuring cortical traits. To follow, we discuss our findings of differential patterns of neuroanatomical changes in tinnitus dependent on our primary variables, namely distress and duration, in the context provided by present knowledge on neural networks that bind together sensation, emotion, and cognition. However, we would like to concede that any interpretation of our CSA and CT findings are constrained by the moderate level of statistical confidence. Furthermore, akin to the previous VBM analysis (Schecklmann et al., 2013), an uncorrected statistical threshold of $p < 0.001$ for the MWC model was deemed feasible due to a lower number of degrees of freedom. Finally, we address the potential limitations and conclude the article with general remarks.

Based on an automated surface-based morphometry approach, the present study delineates neurostructural changes in the brains of individuals suffering from chronic tinnitus. One of the major aims of the study was to test to what extent the SBM approach might provide more nuanced results than the VBM approach. For this reason we re-analyzed the same sample of individuals that had already been analyzed using VBM previously (Schecklmann et al., 2013). While we were able to replicate the changes in bilateral superior temporal cortical volume as already observed by Schecklmann and colleagues, we also demonstrate that the SBM approach enables a more differentiated insight, because it not only allows for the consideration of volume, but also that of the cortical thickness and surface area. Due to this disentangling, we were able to find correlations (uncorrected) that are indicative of distinct relationships in TI between distress and CSA, and duration and CT, respectively.

However, despite the fact that the architecture of SBM makes it possible to investigate more neuroanatomical traits than just CV, the results that it engenders are statistically less reliable. This may be due to the complex interaction of variables in the MWC model with the cortical parameters of CT and CSA, and the mentioned lowered number of degrees of freedom. A competent scholar should be aware of this shortcoming when interpreting the uncorrected data with a threshold of $p < 0.001$, which is not uncommon, as can be seen in the former analysis in the case of the MWC model (Schecklmann et al., 2013), and in similar recent studies (Vanneste et al., 2015).

As indicated by our analysis, CSA and CT can be partly conceived as being distinct traits,

and thus their analysis provides more nuanced information than the sole computation of CV as implemented in VBM. Given this understanding, the present study concurs with a pool of recent reports which also demonstrated the genetically and phenotypically distinctiveness of CT and CSA (Greve et al., 2013; Hogstrom et al., 2013; Koolschijn and Crone, 2013; Koelkebeck et al., 2014; Lyttelton et al., 2009; Meyer et al., 2014a; Storsve et al., 2014; Raznahan et al., 2011; Vuoksimaa et al., 2015; Wierenga et al., 2014). It is important to mention that CV cannot be interpreted as a simple compilation of CT and CSA. With respect to the present study, we suggest a specific understanding of 'distinctiveness' in that there are variations in CSA that relate to distress and (also not) to duration while there are other variations in CT that may also give an account of the measured psychological traits. However, it is important to note that the different variations in CT and CSA do not pertain to the same or overlapping variance in behavior. Accordingly, (Winkler et al., 2010, p. 1141) concluded that "GM volume, which is a composite of 2 other traits (surface area and thickness), might not be the best choice". Even though recent clinical studies (Boyen et al., 2013; Husain et al., 2011; Melcher et al., 2013; Schecklmann et al., 2012; Vanneste et al., 2015) continue to use VBM to identify apparent tinnitus-related group differences, we suggest that surface-based morphometry should be used in complement with VBM because it allows for the computation of three distinct parameters, namely CV, CSA, and CT. Thus, SBM minimizes the risk of underestimating or ignoring existing relationships between CT or CSA and behavior. Hence, the analysis of local differences (in cross-sectional approaches) or changes (in longitudinal approaches) in cortical volume (the arithmetical product of CSA and CT), surface area (the ratio of CV/CT), and thickness (the ratio of CV/CSA) may help to excavate subtle, informative patterns in complex data.

In the context of the comparison between the VBM and the SBM analyses of the same sample of TI, we conclude that our results *prima facie* replicate the outcome of Schecklmann and colleagues in that we also revealed a negative relationship between tinnitus distress and CV in bilateral supratemporal peri-auditory cortical fields. However, more specific, separate analyses of CSA and CT show that a different pattern for those distinct traits can be found in the midportion of the left supratemporal plane, while the less reliable effects in the right temporal lobe are not situated in Heschl's gyrus and, hence, cannot be considered as homologues to the cluster in the left STG (see Figure 2). This discrepant

finding regarding the hemispheres was also observed in the VBM analysis (Schecklmann et al., 2013).

Further into the analysis, we noted a negative relationship between distress and CSA, which means that TI who indicated a higher level of tinnitus-related emotional distress showed a systematically smaller surface of auditory-related cortex. Intuitively, one would expect such a relationship between behavior and CT, as the latter is understood to be a neuroanatomical trait that reflects neuroplastic alterations following enhanced sensory stimulation and experience. The finding that emotional distress, elicited by the sensation of chronic noise, is considerably stronger in individuals who demonstrate a smaller extent of CSA in the core auditory and adjacent insular regions, requires a compelling explanation. The anticorrelation between CSA and distress can be interpreted to imply that CSA, under some circumstances, is sensitive to plastic alterations and may decrease locally. However, with respect to the widely accepted 'radial unit hypothesis' this interpretation is implausible (Rakic, 2000). According to this framework CSA and CT have different origins. While CSA increases during late fetal development due to cortical folding, CT alters dynamically across the entire lifespan as a consequence of training, experience, and disease. By all means, the 'radial unit hypothesis' postulates that changes in CSA and in CT are not causally related to each other; any changes observed in CSA and CT are presumed to reflect different neuronal alterations (Rakic, 2007). Following the assumptions of this hypothesis, one would expect to observe stronger changes in CT of TI relative to CSA, the size of which has been considered to be more static. Alternatively, one may reason that the observed alterations of CSA are not a consequence of tinnitus, but rather reflect a predisposition. A smaller CSA of core auditory and insular regions may predispose increased levels of tinnitus severity. This could be due to a limited cortical capacity for compensation of peripheral hearing loss or general inhibitory functions. A similar observation was made by Schneider and co-authors (Schneider et al., 2009) who investigated the volumetric size of the core auditory regions in TI and CO by means of an in-vivo morphometry study. According to their findings, a reduction of cortical volume in auditory fields might be indicative of a higher predisposition for tinnitus. Even though our result is not this intuitively straightforward to interpret, we consider it to be of value as it provides interesting, additional evidence to the present debate about the roles of CT and CSA in pathology-related neuroplastic alterations. Longitudinal studies would

be needed to answer the question whether the observed changes reflect predisposition or consequence of tinnitus, and to reveal the involved mechanisms. This is not a trivial issue as the results of current and recent functional and structural brain imaging studies are inconsistent regarding the functional or structural changes in the auditory cortex related to tinnitus (Adjamian et al., 2014). While one recent study reported an increase in GM in the left primary auditory cortex of tinnitus patients (Boyen et al., 2013), other studies observed a volume decrease in auditory regions (Aldhafeeri et al., 2012; Schecklmann et al., 2013; Schneider et al., 2009). To account for these differences it is reasonable to consider the role of duration as in our data we find differential neuroplastic alterations in the left auditory regions for distress and duration. Tinnitus duration is positively correlated with CT in adjacent portions of the temporal lobe whereas tinnitus distress is anticorrelated to CV and CSA in auditory regions. To overcome the unsatisfactory condition of inconsistency between different tinnitus-related studies, we consider it of the utmost importance to perform careful analyses on existing datasets in order to confirm previous results with differential analysis techniques, rather than the collecting and analyzing of new data. The present study should be considered a convenient example towards the realization of this strategy, and it is in line with concurrent efforts and suggestions forwarded by globally operating initiatives such as TINNET (http://tinnet.tinnitusresearch.net/).

We were not surprised to notice a relationship between distress and insular morphology as this sub-sylvian area can be conceived as an interface between the auditory system (Bamiou et al., 2003; Mutschler et al., 2009) and the emotional brain circuitry (Yao et al., 2016) in the human brain. Along the same lines, Leaver and coworkers (Leaver et al., 2012) observed a positive relationship between tinnitus distress and CT in the left anterior insula. Despite the fact that we report uncorrected results, we think it is appropriate to discuss our findings in light of present knowledge, as other scholars have also established a tight link between tinnitus and the insular region (Leaver et al., 2012; Moazami-Goudarzi et al., 2010; Van der Loo et al., 2011). With reference to the tinnitus network, the insula has frequently been nominated as a key component due to its unique positioning, which facilitates integration across multiple domains including social, emotional, and attentional systems (Chiarello et al., 2013). According to Nieuwenhuys and colleagues (Nieuwenhuys, 2012), the anterior insula subserves a multitude of functions, including the perception of pain and introspection about feelings. Other authors emphasize the involvement of

the anterior insula in a network that is related to salience detection (Cauda et al., 2012). In conjunction with the ACC, the anterior insula "forms the core of a salience network that facilitates the detection of important environmental stimuli" (Menon and Uddin, 2010, p. 663) and thus can be conceived as an "integral hub in mediating dynamic interactions between other large-scale brain networks involved in externally oriented attention and internally oriented or self-related cognition" (Menon and Uddin, 2010, p. 655). Furthermore, the tandem of anterior insula and ACC "integrate[s] bottom-up attention switching with top-down control and biasing of sensory input" (Menon and Uddin, 2010, p. 663). Given all this evidence, it is plausible that the observed subtle correlation between morphological alterations of the anterior cingulate-insular circuit and tinnitus distress may reflect an inappropriate evaluation of internally generated sounds and detrimental loss of inhibitory attentional control.

For the relationship between CT and distress, we observed a positive correlation (uncorrected) in the bilateral posterior cingulate while CSA was negatively correlated (uncorrected). A similar relationship was not observed by Schecklmann and colleagues (Schecklmann et al., 2013) and can thus be considered an additional value of our SBM analysis. It is an example of a 'canceling-out' effect in CV as CT and CSA are both positively and negatively correlated with distress in the same region, and therefore changes in CV may not be discernible. Furthermore, the findings at this site are in accordance with the predictions based on the 'radial unit hypothesis' in that CT is typically regarded as a neuroanatomical trait that may increase or decrease as a function of lifespan development and disease. In the context of the present pattern of results, this finding indicates that an increase in distress may result in an increase of the synaptic connectivity and neuronal density (CT) in the posterior cingulate cortex, whereas the smaller CSA could be an underlying predisposition for this change in CT. Even though our reasoning is based on uncorrected results, we consider it relevant to report both the results and the reasoning, because they provide support for current models of tinnitus circuits in the human brain. In line with the framework described by De Ridder and coauthors (De Ridder et al., 2014), the posterior cingulate cortex is a node of the large-scale neural network that represents tinnitus-related distress. Jastreboff (Jastreboff, 2011) located memory-related functions in the context of tinnitus to the posterior cingulate. With respect to the proposal by De Ridder and colleagues (De Ridder et al., 2011a) these results can be easily reconciled. Ac-

cordingly, tinnitus emerges as a function of several large-scale networks that bind together various aspects of perception, salience, memory, distress, and audition. The cingulate cortex appears to play a key role in these large scale networks as we observed both increases and decreases of CT and CSA in distinct portions of the cingulate cortex with tinnitus distress.

In addition, we analyzed the relationship between duration and changes in CT and CSA, respectively. Notably, a recent study that combined EEG with VBM analyses in a large sample of TI (Vanneste et al., 2015) failed to discover any relationship between CV and duration (even though they applied the same vertex/voxel-wise significance threshold that we used in our analysis). While in our sample no effects were observed for CSA, we did find two significant effects for CT (uncorrected). The first effect is an anticorrelation between CT in the subgenual anterior cingulate cortex (see Figure 5). This indicates that the longer the duration of tinnitus, the stronger the morphological reduction in this region (or vice versa). Interestingly, two recent frameworks consider this region to be of key importance in the pathophysiology of tinnitus. According to the 'phantom pain' model by De Ridder and colleagues (De Ridder et al., 2011a), "the subgenual anterior cingulate cortex mediates an overlap (or hub) with a central autonomic control system" (Adjamian et al., 2014, p. 123). Furthermore, Vanneste and colleagues (Vanneste et al., 2010, p. 478) linked the subcallosal anterior cingulate cortex to a circuit that is part of a "common emotional and attentional distress network". However, our analysis provided no evidence for a link between the subcallosal area and tinnitus-related distress.

It is our view that the 'gating' model by Rauschecker and colleagues (Rauschecker et al., 2010) provides an alternate and more plausible explanation. According to this model, tinnitus is the result of a dysfunction in a cortical-subcallosal-thalamic loop. In non-affected individuals this circuit functions as a system that tones down unwanted auditory noise, in that serotonergic neuronal ensembles in the subcallosal ventral striatum modulate the function of the thalamic reticular nucleus (TRN). The TRN is meant to inhibit the auditory thalamus and, in so doing, to block the aversive sound from encountering the auditory cortex. In the terminology of Rauschecker and colleagues (Rauschecker et al., 2010) the mechanism can be termed a 'tuning out' device that filters out the tinnitus signal. The model further proposes that in TI the integrity of the subcallosal circuit is disrupted, in which case the inhibiting projections of the TRN to the auditory thalamus are attenuated,

and hence the tinnitus percept is relayed to the auditory cortex without hindrance. According to this model, anomalies within this limbic-cortico-striatal-thalamic loop result in deviant processing of the tinnitus sound. Due to the gating mechanism breaking down, the inhibitory device that is part of a normal noise canceling system does not work properly. Rauschecker et al. (Rauschecker et al., 2010, p. 823) propose that the "inhibition of the tinnitus signal at the thalamic gate is lost". As such the persistent sound becomes salient and "leads to permanent reorganization and chronic tinnitus" (Rauschecker et al., 2010, p. 823). It is plausible to reason that a steadily progressive long-term thinning of the subcallosal area fosters the establishment of this vicious cycle. Remarkably, our finding of an anticorrelation between duration and CT in the subcallosal area (even though it is based on uncorrected results) fully concurs with the concept that ongoing tinnitus may be related to progressive reorganistion of the subcallosal anterior cingulate, which is in line with the 'gating' model (Rauschecker et al., 2010), but would require a modification of the 'phantom pain' model. A similar finding has been reported by Leaver et al. (Leaver et al., 2012) who also applied the SBM approach to identify neuroanatomical markers of tinnitus. Notably, Leaver and colleagues obtained their results by comparing TI with controls who were matched for age and hearing loss. Although this makes it difficult to compare their results with the current findings, some observations merit discussion here. Similar to our results Leaver and co-authors also noted a decrease in CT in the subcallosal area. However, whereas Leaver and colleagues excavated a relationship between CT decrease and increased depression and anxiety scores, we observed a subcallosal decrease related to duration when data were corrected for BDI ($-log10(p) = -3.37$, $p < 0.001$)[2]. We have therefore concluded that tinnitus duration is the sole factor, independent of any other factor, that results in a decrease of thickness in the subcallosal area.

A converse effect (i.e., an uncorrected positive correlation between CT and duration) was evident in the left anterior superior temporal lobe. The significant cluster covers a strip of cortical tissue at the lateral surface convexity of the STG, extending to the STS. This part of the human brain cannot be considered a part of the core tinnitus network in a strict sense, even though De Ridder and colleagues involve the left superior temporal lobe in their reasoning (De Ridder et al., 2014). Based on a study by Brancucci and co-workers (Brancucci et al., 2011), De Ridder and colleagues mention that the bilateral middle tem-

[2]In our sample Beck Depression Inventory data (BDI, (Beck et al., 1961)) for 154 patients are available

poral gyrus is part of a subnetwork that mediates the awareness of pitch. Elsewhere in the same paper, De Ridder and co-authors discuss the left anterior STS as being a part of a subnetwork that serves self-perceptional functions. They reason that awareness and self-perception are densely intertwined. Hence, the self-perception network (in connection with the salience network involving the anterior insula and the dorsal anterior cingulate cortex) "most likely has to be activated for the tinnitus to be consciously perceived" (De Ridder et al., 2014, p. 20). We favorably interpret the CT increase in the left anterior STG/STS as a plastic effect that may have resulted from the increased awareness and self-perception in TI.

Interestingly, the Freesurfer analysis also revealed alteration in subcortical structures even though the software is optimized for cortical surface measurements. For this reason we are reluctant to interpret these findings. However, the subcortical nuclei, namely the thalamus and the amygdala, have also already been located roles in tinnitus-related networks (De Ridder et al., 2014). Regarding the thalamus, it is additionally worth mentioning that it is part of a triangle, consisting of auditory cortex, insula and thalamic nuclei, that supports audition in general (Meyer et al., 2003).

General remarks and limitations

Our reasoning may help to capture innovative aspects of the causal interplay between observed neuroanatomical changes and behavioral patterns in a large sample of TI. Although some of our conclusions are based on uncorrected results, we consider it relevant to report them transparently, because they can be seen as pieces of a mosaic that either provide evidence for or against the current frameworks of tinnitus generation and maintenance. In addition to the major results reported and discussed above, several minor issues that might be of interest are communicated in the following.

One objection may be that the current study does not involve a control group that consists of individuals without symptoms of tinnitus. First, our aim was to replicate and to extend the results reported by Schecklmann and coworkers. Their previous study did not include a control group either. Second, we focused on the relationship between tinnitus-related behavioral parameters, namely distress and duration, and neuroanatomical alterations. Recent studies comparing neuroanatomical differences between TI and normal controls reported inconsistent results. We think that the considerable variance within the samples

of TI accounts for these partly contradictory findings. For this reason we refrained from adding a group of controls. Furthermore and with respect to future studies, we advocate a change in tinnitus research paradigms in that we propose that the various facets of tinnitus should primarily be investigated within large samples of TI rather than contrasting TI with non-affected controls.

Another question may be why we did not observe a relationship between neuroanatomical changes and hearing loss. Again, we would like to emphasize that the statistical models we applied in the analysis were controlled for hearing loss. The same holds for the lateralization of tinnitus.

Current and recent functional and structural brain imaging studies are inconsistent pertaining to the relationship between self-reported tinnitus laterality and functional or structural changes in the auditory cortex (Schneider et al., 2009). In the present study, the majority of TI indicated a bilateral tinnitus experience (n=182), which would suggest that bilateral neuroanatomical changes in this region would be found. However, we only noted a significant negative relationship between CSA and distress in the left, but not in the right, core auditory region in the ROI analysis (see Figure 3). At the very least, our data partly concur with the observations from a recent functional study (Geven et al., 2014), in that the tinnitus-related activation appears to be left-dominant (whereas activation in the right hemisphere is more wide-spread) and independent of self-reported tinnitus laterality. Akin to the study of Vanneste et al. (Vanneste et al., 2015), we did not obtain significant differences for neuroanatomical measurements by contrasting unilaterally distributed (left vs. right) TI sub-samples. Regarding future studies, we recommend that the potential influence of hearing loss and tinnitus lateralization be considered when the study is designed in order to investigate specific hypotheses and respective contrast groups with a sufficient number of cases.

Our finding of a decrease in the subcallosal thickness related to tinnitus duration may be explained by the limited range of available audiometry. According to Melcher and co-authors (Melcher et al., 2013), high-frequency hearing loss (>8KHz) but not tinnitus per se may account for reductions in subcallosal gray matter.

Naturally, a neuroanatomical examination that considers only gray matter traits is not complete. We agree that changes in white matter architecture can also be conceived as neuroplastic biomarkers of the tinnitus network (Aldhafeeri et al., 2012; Crippa et al.,

2010; Husain et al., 2011). As outlined by Adjamian and co-authors (Adjamian et al., 2014) these studies report both local increases and decreases of white matter fiber tracts in TI. However, it cannot be ruled out that this "evidence more consistently suggests that hearing loss induces white matter alterations, and when taking this into account differences related to tinnitus prove debatable" (Adjamian et al., 2014, p. 129). Unfortunately, diffusion-weighted imaging (DWI) recordings of our sample of TI are not available, and we are therefore not able to provide complementary evidence on altered white matter architecture.

Albeit that our study revealed that distinct cortical areas clearly vary systematically in surface or thickness as a function of tinnitus-related distress and duration, we lack the evidentiary basis from which to conceive these ensembles of areas as 'networks' in a strict sense. To learn more about the existence of tinnitus-related neural subnetworks as delineated by DeRidder and et al. (De Ridder et al., 2014), it is not sufficient simply to expose distinct areas without a complementary analysis of effective structural connectivity. To this end, Golm and collaborators (Golm et al., 2013) raise the question of how tinnitus-specific a 'network' made up of a sample of typical candidate regions may indeed be?

Yet another question raised may be whether an increase or a decrease of CT should be considered advantageous or detrimental. Intuitively, it is reasonable that a thicker cortex would accommodate more neuronal packing and would allow for more computational resources, thus making it conceptually more proficient. However, the conclusion that a 'thicker' cortex can generally be considered a 'better' cortex is not sanctioned by the literature. The question as to what extent a 'thicker' cortex is 'better' can only be discussed when it is carefully embedded in the context around each study-specific dataset (Meyer et al., 2014a).

The important issue of direction of causality is often ignored in cross-sectional studies that seek to establish relationships between specific behavioral traits and neuromorphological changes. According to the standard approach, it is assumed that increased distress or annoyance, which appears to be related to the occurrence and maintenance of tinnitus, causes structural changes in the tinnitus brain. However, one cannot rule out the opposite causal relationship in which smaller/larger brain regions cause the pathological behavior, namely tinnitus. Longitudinal studies are arguably the sole approach towards ending this debate.

Overall, like the previous VBM analysis (Schecklmann et al., 2013) and indeed neuroanatomical studies on tinnitus in general, the statistical effects here are small and reported transparently. Furthermore, we refrained from reporting results which were not comparable with the statistical procedure of the former VBM analysis.

The reanalysis of a large sample of thoroughly investigated TI with an innovative approach is one beneficial aspect of the current study. Thus, we are able to directly compare the pros and cons of the two analysis techniques, while all other factors (scanning environment, participants' profiles and neuroanatomical heterogeneity) are strictly controlled. Based on comprehensive psychometric protocols of the participants involved in the two studies, a multitude of post-hoc computations are possible. With respect to future studies, the establishment of large databases, longitudinal designs, and the introduction of homogeneous procedures of data analysis should be considered both convenient and imperative. Furthermore, the combined use of functional/structural imaging techniques (MRI, diffusion weighted imaging, EEG/MEG) as recently published by Vanneste et al. (Vanneste et al., 2015) should be established as a second important line of development.

Conclusion

By applying an automatic standardized SBM data analysis approach, namely FreeSurfer, we were able to extend the results of a previous VBM study on the same sample of TI. In more detail, our approach identified specific relationships between behavioral tinnitus-related parameters and distinct neuroanatomical traits, namely CT and CSA. Based on uncorrected results, we observed that tinnitus distress seems to be favorably related to a reduction in CSA, while tinnitus duration appears to correspond to changes in CT. Hence, SBM as compared to VBM seems to generate a wider array of results which may allow for a more nuanced insight into the subtle cortical neurodynamics of tinnitus.

Despite the recently expressed reservations on the present progress of our understanding of the relationship between tinnitus and gray matter alterations (Adjamian et al., 2014; Elgoyhen et al., 2015), we consider the present study to be a valuable and necessary contribution towards reconciling recent technical and methodological advancements.

Acknowledgements:

This research was supported by the 'Fonds zur Förderung des akademischen Nachwuchses' (FAN) des 'Zürcher Universitätsvereins' (ZUNIV), by TINNET - COST Action BM1306 'Better Understanding the Heterogeneity of Tinnitus to Improve and Develop New Treatments' and by the University Research Priority Program 'Dynamics of Healthy Aging' of the University of Zurich. During the study and preparation of publication Patrick Neff was sponsored by 'Stiftung fuer wissenschaftliche Forschung an der Universität Zürich'. We are indebted to Allison Christen and two anonymous reviewers for their exceptionally helpful comments on this manuscript.

Author contributions:

MM: substantial contribution to the design of the study, drafted and revised the manuscript. PN: substantial contribution to the design of the study, data analysis, drafted and revised the manuscript. FL: substantial contribution to the design of the study, revised the manuscript. TK and SW: critically revised the manuscript. MS and BL: substantial contribution to the design of the study, data acquisition, critically revised the manuscript.

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2.2 10 Hz amplitude modulated sounds induce short-term tinnitus suppression

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A similar version of this manuscript has been published in Frontiers in Aging Neuroscience, May 2017, Volume 9, Article 130. DOI: 10.3389/fnagi.2017.00130.

Abstract

Acoustic stimulation or sound therapy is proposed as a main treatment option for chronic subjective tinnitus. To further probe the field of acoustic stimulations for tinnitus therapy, this exploratory study compared 10 Hz amplitude modulated (AM) sounds (two pure tones, noise, music and frequency modulated (FM) sounds) and unmodulated sounds (pure tone, noise) regarding their temporary suppression of tinnitus loudness. First, it was hypothesized that modulated sounds elicit larger temporary loudness suppression (residual inhibition) than unmodulated sounds. Second, with manipulation of stimulus loudness and duration of the modulated sounds weaker or stronger effects of loudness suppression were expected, respectively.

We recruited 29 participants with chronic tonal tinnitus from the multidisciplinary Tinnitus Clinic of the University of Regensburg. Participants underwent audiometric, psychometric and tinnitus pitch matching assessments followed by an acoustic stimulation experiment with a tinnitus loudness growth paradigm. In a first block participants were stimulated with all of the sounds for 3 minutes each and rated their subjective tinnitus loudness to the pre-stimulus loudness every 30 seconds after stimulus offset. The same procedure was deployed in the second block with the pure tone AM stimuli matched to the tinnitus frequency, manipulated in length (6 minutes), and loudness (reduced by 30 dB and linear fade out). Repeated measures mixed model analyses of variance (ANOVA) were calculated to assess differences in loudness growth between the stimuli for each block separately.

First, we found that all sounds elicit a short-term suppression of tinnitus loudness (seconds to minutes) with strongest suppression right after stimulus offset ($F(6,1331)=3.74$, $p<0.01$). Second, similar to previous findings we found that AM sounds near the tinnitus frequency produce significantly stronger tinnitus loudness suppression than noise (vs.

Pink noise: t(27)=-4.22, p<0.0001). Finally, variants of the AM sound matched to the tinnitus frequency reduced in sound level resulted in less suppression while there was no difference observed for a longer stimulation duration. Moreover, feasibility of the overall procedure could be confirmed as scores of both tinnitus loudness and questionnaires were lower after the experiment (tinnitus loudness: t(27)=2.77, p<0.01); Tinnitus Questionnaire: t(27)=2.06, p<0.05; Tinnitus Handicap Inventory: t(27)=1.92, p=0.065).
Taken together, these results imply that AM sounds, especially in or around the tinnitus frequency, may induce larger suppression than unmodulated sounds. Future studies should thus evaluate this approach in longitudinal studies and real life settings. Furthermore, the putative neural relation of these sound stimuli with a modulation rate in the EEG α band to the observed tinnitus suppression should be probed with respective neurophysiological methods.

Introduction

Subjective tinnitus is defined as 'the perception of sound(s) in the absence of an external sound source' (Erlandsson and Dauman, 2013; Eggermont and Roberts, 2004) and is deemed chronic after 12 months since first occurrence (Mazurek et al., 2010). No less than 35 percent of the general (US) population are haunted by this phantom auditory perception at some point during their lifetime (Jastreboff, 1990). 10-15 percent report their tinnitus percept as being frequent or continuous and approximately 1-2 percent suffer heavily under the condition (Langguth et al., 2013). With a steadily aging demographic, tinnitus is becoming increasingly prevalent and relevant (Hoffman and Reed, 2004; Nondahl et al., 2012). Besides the tantalizing phantom sound or comorbidities like depression, stress and anxiety (Langguth et al., 2013), tinnitus also impacts daily life functions in healthy aging as impaired hearing, sound localization and speech perception can lower the quality of life in tinnitus sufferers (Moon et al., 2015; Hyvärinen et al., 2016; Gilles et al., 2016).

In the majority of cases tinnitus manifests as a single tone, ringing or noise with a definable pitch and loudness, which is perceived bilaterally or with a slight preference to one side, or alternatively lateralized to one ear (Lockwood et al., 2002). Tinnitus pitch, laterality and loudness can be therefore considered as the main (subjective) perceptual parameters of interest in addition to maskability and residual inhibition by external sounds (Henry and Meikle, 2000). Usually, tinnitus is considered to be caused by either objective

(Eggermont and Roberts, 2004; Mazurek et al., 2010; Schaette and Kempter, 2006) or hidden hearing loss (Schaette and McAlpine, 2011; Weisz et al., 2006; Adjamian et al., 2012; Xiong et al., 2013), where loss of cochlear hair cells in objective hearing loss has been shown to lead to maladaptive plasticity throughout the auditory pathway and brain. Tinnitus pitch seems to average near the frequency of maximal hearing loss, especially in sufferers with pure-tone tinnitus (Schecklmann et al., 2012). Related to this maladaptive plasticity, a similarity of tinnitus to phantom limb or general phantom (pain) perception following sensory deafferentation has also been proposed (De Ridder et al., 2011). Although models of pathogenesis and physiology are still being debated and are limited by an underlying inherent heterogeneity of the disorder, it can be stated with confidence that both the inner ear and the brain are involved (Elgoyhen et al., 2015; De Ridder et al., 2014; Vanneste and De Ridder, 2012; Eggermont and Roberts, 2004; Jastreboff, 1990; Adjamian et al., 2009; De Ridder et al., 2011).

Acoustic stimulations have been used in various forms to counteract or alleviate the malicious phantom percept (Jastreboff, 2007). From a clinical routine perspective, acoustic stimulation or sound therapies are proposed as symptom-oriented treatment options besides cognitive behavioural therapy and neuromodulation or -stimulation if chronic subjective tinnitus persists after standard clinical assessment and intervention (Langguth et al., 2013). Traditionally, masking approaches using broadband or narrow-band noise, or pure tones, were established first (Feldmann, 1971; Vernon, 1977; Hazell and Wood, 2009; Henry et al., 2004; Watanabe et al., 1997). These maskers have also been administered in hearing aids (Vernon and Meikle, 2003) with slightly better effects than hearing aids without maskers as shown in a study by Henry et al. (2015). In recent times, two major acoustic stimulation techniques for long-term, daily intervention have been developed building on the model of lateral inhibition (Pantev et al., 2012; Adamchic et al., 2014). Following peripheral hearing loss, central tonotopic map reorganization and hyperactivity in regions of the reorganization responsible for the tinnitus sensation (Eggermont and Komiya, 2000; Eggermont and Roberts, 2004), lateral inhibition is theorized to counteract or reverse this maladaptive hyperactivity. Pantev and colleagues therefore proposed to apply a notch filter in a single octave band around the tinnitus frequency to music. The energy of the sound signal at the edges of the notch filter is theorized to inhibit the frequencies around the tinnitus pitch therefore reversing the maladaptive plasticity, which

has been shown to be effective in long-term intervention (Okamoto et al., 2010). The width of the notch filter did not significantly influence treatment effects in a further study (Wunderlich et al., 2015b) while the spectral contrast (i.e. increased sound pressure at frequencies neighboring the filter edges) seems to improve the treatment effects as shown in a further follow up study (Stein et al., 2015). Building on similar reasoning about frequencies neighboring the tinnitus pitch and lateral inhibition, Tass and colleagues (Tass et al., 2012) established a method where sine tones are presented in a randomized fashion around the tinnitus frequency for several hours a day with similar longitudinal therapeutic effects.

While the established approaches focus on the retraining of auditory and related cortical structures in longitudinal therapeutic interventions (Adamchic et al., 2014; Pantev et al., 2012), only few studies looked at the effect of sounds on the temporary suppression of tinnitus (Roberts et al., 2006, 2008; Reavis et al., 2012) to identify possible candidates for future tinnitus sound therapies. Acoustic stimulation with amplitude modulation (AM) and frequency modulation (FM) (Reavis et al., 2012; Tyler et al., 2014) has just recently entered this line of research building on results of electrical stimulation of the cochlea (Zeng et al., 2011). The results of these studies indicate that especially AM sounds in the higher, tinnitus-relevant frequencies of 3000-9000 Hz produce a more pronounced tinnitus suppression during and after the stimulation compared to their unmodulated pendants or white noise. In any case, longitudinal data on efficacy and long-term as well as momentary neuroplastic alterations of continuous modulated or patterned, sounds is missing. Therefore, approaches showing efficacy and feasibility in single session experiments with short stimulation duration measuring tinnitus suppression (i.e. residual inhibition) should be tested in longitudinal, prospective placebo-controlled studies to assess long-term efficacy. While recent studies with AM and/or FM sounds, used 40 Hz for the modulation rate (Reavis et al., 2012; Tyler et al., 2014), which is known to produce the largest neural responses in auditory cortex through entrainment as shown in auditory steady-state response (ASSR) paradigms (Picton et al., 2003), no former study tested the influence of lower modulation rates in different carrier sounds, including the tinnitus pitch, for tinnitus suppression and therapy. Of special interest here, several reviewed studies in Picton et al. (2003) could also show entrainment effects for different bands including the alpha frequency band. Cortical auditory α activity has been shown to be decreased in tinnitus

patients in MEG (Weisz et al., 2005; Schlee et al., 2014), EEG (Moazami-Goudarzi et al., 2010) and possibly also reduced in variability (Schlee et al., 2014). Looking at modulation depth of the stimuli and strength of (entrainment) effect as measured by EEG or MEG, several studies have reliably shown entrainment effects of monaural AM stimuli (100% modulation depth) superior to binaural AM stimuli (Picton et al., 2003; Becher et al., 2014; Draganova et al., 2008; Schwarz and Taylor, 2005). A modulation rate in the α frequency band as well as monaural stimuli with a maximized entrainment effect may therefore enable a normalization of reduced auditory α and thereby concomitantly reduce the tinnitus percept. Based on this preliminary reasoning we here investigated the effects of AM sounds in the α band for tinnitus sound therapy. Yet, the focus of this study was set on the behavioral level to proof the concept and feasibility in the absence of neurophysiological methods.

In the exploratory study at hand, we therefore tested the influence of 10 Hz AM sounds (two pure tones, noise, music and FM sounds) and unmodulated sounds (pure tone, noise) on the temporary suppression of subjective tinnitus loudness in participants with tonal tinnitus in block 1 of the experiment. We hypothesize that all sounds may elicit a short-term suppression of tinnitus loudness (seconds to minutes) with strongest suppression right after stimulus offset (Roberts et al., 2006, 2008; Reavis et al., 2012; Tyler et al., 2014). Given the different types of modulated and unmodulated sounds with frequencies in or around the actual tinnitus pitch, we expect to find differential suppression patterns between the stimuli with AM sounds possibly eliciting enhanced suppression (Reavis et al., 2012). Additionally, with the manipulation of stimulation length and loudness in block 2 of the experiment, we anticipate more pronounced or weaker effects of tinnitus loudness suppression, respectively.

Methods

Participants

Patients with chronic tonal tinnitus (>12 months tinnitus duration), who had consulted the multidisciplinary Tinnitus Clinic of the University of Regensburg, were included in the study if their age was between 18 to 75 years. Patients with history or presence of severe and relevant somatic, neurological, or mental disorders were excluded. Intake of psychotropic medication or ongoing participation in tinnitus therapies were further

exclusion criteria. The study was approved by the Ethics Committee of the University of Regensburg (16-101-0061). All participants gave written informed consent after a comprehensive explanation of the procedures.

After signing the consent form all participants completed the tinnitus questionnaire (TQ) (Goebel and Hiller, 1994), the Tinnitus Handicap Inventory (THI) (Newman et al., 1996), and a visual analog scale (VAS) (Adamchic et al., 2012) with respect to tinnitus loudness (spanning from inaudibility to maximal imaginable loudness). The Tinnitus Sample Case History Questionnaire (TSCHQ) was used to gather clinical and demographic data of all patients (Langguth et al., 2007). Furthermore, hearing level was measured with a standard audiogram using frequencies ranging from 125 Hz to 8 kHz in octave steps with semi-octave steps between 2 and 4 (i.e. 3 kHz), and 4 and 8 kHz (i.e. 6 kHz), respectively (Madsen Midimate 622D; GN Otometrics, Denmark). Headphones used for audiometry, tinnitus matching, as well as for the stimulation procedure were quasi-linear in their frequency response over the whole audible spectrum (Sennheiser HDA 2000; Sennheiser, Germany).

Questionnaire scores and participants characteristics are listed in table 2.8. The distribution of sexes in the sample was slightly skewed with 11 female and 18 male participants. 3 participants reported a purely left-sided, 2 participants a purely right-sided tinnitus. The majority of participants indicated some form of bilateral or diffuse tinnitus location, with 8 participants indicating tinnitus in both ears, 4 inside the head, 7 both ears with a tendency to the left side, and 4 with a tendency to the right side. A specific tinnitus laterality was not considered as an inclusion criterion due to the diotic presentation of the stimuli. Hearing thresholds slightly differed between ears (right side: mean=40.63, SD=13.24; left side: mean=39.46, SD=12.17; $t(28)=2.10$, $p=0.044$).

Tinnitus matching

After filling in the questionnaires and audiometry, participants were seated in front of a screen with a computer mouse and instructed for the tinnitus matching via software. The matching procedure was designed around a sine tone generator (Meyer et al., 2014) where pitch (in single Hz resolution), amplitude and laterality (panning) could be defined and controlled using MAX software (MAX 7; Cycling'74, USA). First, the loudness and lateralization of the tinnitus was roughly defined followed by the actual pitch by the study

	Mean	SD[a]	Median	Minimum	Maximum
Age (years)	52.34	12.78	54	24	75
Tinnitus duration (months)	123.66	117.74	71	12	431
Hearing Loss (both ears, dB)	38.29	11.78	37.27	15.91	62.73
TQ[b] total score (0-84)	39.41	14.06	40	10	69
THI[c] total score (0-100)	43.97	18.48	44	10	92
Tinnitus loudness (%)	67.59	14.74	70	30	100
VAS[d] loudness (0-100)	54.93	17.26	55	22	86
Tinnitus awareness (%)	66.55	26.73	60	0	100
Tinnitus frequency (matching, Hz)	5334.77	2904.96	6000	911	10500
Tinnitus loudness (matching, dBA)	45.46	14.92	43.90	23.50	81.60

Table 2.8: **Participants Characteristics (n=29)**. [a]SD = Standard Deviation. [b]TQ = Tinnitus Questionnaire (Goebel and Hiller, 1994). [c]THI = Tinnitus Handicap Inventory (Newman et al., 1996). [d]VAS = visual analog scale.

personnel (Penner and Bilger, 1992; Henry and Meikle, 2000). Participants were then made comfortable with the handling of the pitch dial on the graphical user interface and informed about the possibility to adjust the tinnitus pitch in 1 Hz steps while holding down the shift key on the keyboard. Following that, participants proceeded with the actual pitch matching self-reliantly. To ensure reliability and validity of the procedure, the final pitch indicated by the participant was shifted an octave down and up and checked with the participant, respectively, to control for possible octave confusion. Finally, the matched tone was evaluated in a short discussion with the study personnel and rated on a 5 point likert scale (1 = not at all matching the tinnitus percept, 5 = perfect fit). Frequency and loudness results of the matching procedure are listed in table 2.8.

Sound stimuli

A set of 3 amplitude modulated, 2 notch filter amplitude modulated as well as 2 unmodulated sounds were prepared in MATLAB (Matlab R2015a; Mathworks, USA). Besides sine tones in 4 and noise in 2 conditions, a variety of popular music songs was provided to the participants out of which they could choose their favourite song for (notch filter modulated) presentation in one condition. A sum total of 7 acoustic stimuli or conditions with 3 minutes of duration was therefore produced for each participant for block 1. In the remainder of this manuscript, including tables and figures, we termed the different stimuli as follows: 'AMTinnitus' for AM sounds centered at the tinnitus frequency (figure 2.6,

panel A), 'Tinnitus pure tone' for unmodulated sounds centered at the tinnitus frequency (figure 2.6, panel B), 'AMFM' for the AM FM sound (figure2.6, panel C), 'AMLow' for AM of the 108 Hz sound (figure 2.6, panel D), 'AMMusic' for the AM of musical songs (figure 2.6, panel E), 'AMPinknotch' for the filter AM of pink noise (figure 2.6, Panel F), and 'Pink noise' for the pink noise sound (figure 2.6, panel G). For block 2, participants could choose their favourite stimulus, besides AM in the tinnitus frequency (AMTinnitus), after completing block 1. The AMTinnitus and the chosen stimulus were then manipulated in length, or loudness, or faded out (linear fade out in the last minute of the stimulus) resulting in 3 conditions for two stimuli in block 2.

For AMTinnitus, a carrier sine tone was generated and amplitude modulated (100% modulation depth) with a sinusoidal function according to the following principle, where the first factor of the arithmetic product represents the carrier sound and the second factor the modulator. Note that the information in brackets in the legend of the formula is indicative of study-specific settings:

$$s = ca * \sin(2 * \pi * cf * t) * mia * \cos(2 * \pi * mf * t + \phi) \tag{2.1}$$

where:

s sinusoidally amplitude modulated sound

ca carrier amplitude

cf carrier frequency (=tinnitus frequency)

t time

mia modulator index/amplitude (=1)

mf modulator frequency (=10 Hz)

ϕ phase

For the AMPinknotch and AMMusic sounds the target of the 10 Hz modulator was the notch filter amplitude. The notch filter used (Butterworth, filter order = 4) was centered around the matched tinnitus frequency with a filter bandwidth of 1 octave (Okamoto et al., 2010; Wunderlich et al., 2015a). With the filter amplitude modulation applied the resulting sounds where rhythmically suppressed in the octave around the tinnitus frequency giving the acoustic impression of a slight flutter in the stimulus.

For the AMFM sound, a FM sweep from 0 Hz up to the tinnitus frequency with a modulation rate of 10 Hz served as the carrier sound, which was then amplitude modulated like AMTinnitus (i.e. 100% modulation depth). AMLow with a low frequency carrier

sound (108 Hz instead of tinnitus frequency) was generated analogously to AMTinnitus. Finally, the unmodulated stimuli, namely Tinnitus pure tone and Pink noise, were generated. Possible transient artifacts were avoided in the beginning and the end of the stimuli through ramping (linear fade with 100 ms window). Stimuli were then normalized in sound level and finally exported for the experimental procedure.

Acoustic stimulation procedure

All stimuli were presented at sound levels of 60 dB SL in block 1 (i.e. in broadband stimuli noise and music to the average hearing threshold, whereas in frequency specific stimuli the nearest frequency of the audiogram was chosen as reference for the level adjustment). For block 2, the AMTinnitus and the stimulus of choice were 1) presented for 6 minutes, 2) reduced in sound level (30 instead of 60 dB SL) and 3) processed with a linear sound level fade out in the last minute of the stimulus. By varying these core parameters of stimulation length and sound level in block 2, we wanted to test differential tinnitus suppression patterns within single stimuli classes with a focus on AMTinnitus. To ensure comfort and safety of the participants, 80dBA was the upper limit for the sound level of all stimuli. Sound level was carefully checked with an SPL meter (NTi Audio XL2; NTi Audio, Lichtenstein) before actual stimulation. Participants were reminded of the option to interrupt the procedure whenever a sound was deemed uncomfortable at any point of the experiment.

For the acoustic stimulation procedure participants were seated comfortable facing a window with a view on trees to avoid distraction and ensure calmness. No particular instruction was given to focus their attention on either the sound or tinnitus. The presentation sequence of the stimuli was randomized in the two blocks for each participant. Participants were instructed to relax during the acoustic stimulation and to rate the loudness of their tinnitus in percent, compared to the pre-stimulation loudness, after each stimulation at time points 0, 30, 60, 90, 120, 150, and 180 seconds. A similar approach of tinnitus loudness growth was used in the study by Reavis and colleagues (Reavis et al., 2012). However, we diverged from the former study by not measuring suppression during acoustic stimulation, having no reference tones in and after the stimulation and deploying a loudness regime tied to hearing loss with 60 dB SL (Reavis et al. (2012) presented stimuli slightly below matched tinnitus loudness). There was a short break between the blocks

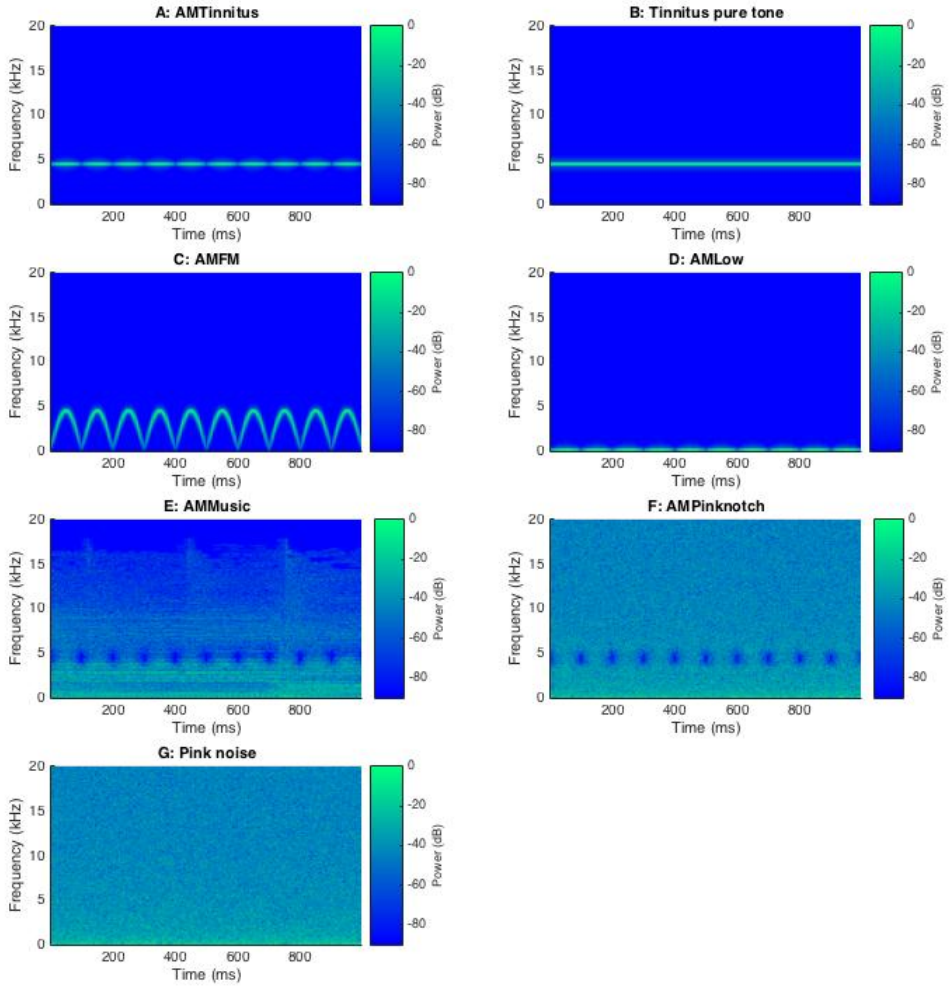

Figure 2.6: **Spectrograms of all sound stimuli (1 second snippets)**. For all of the plotted representative stimuli an arbitrary tinnitus frequency of 4500 Hz was chosen and stimuli normalized to full digital displacement. The modulation rate was constant at 10 Hz in modulated sounds (Panels A, C, D, E, F) whereas Panels B and G represent the unmodulated stimuli. Stimulus presentation was set to 3 minutes for all stimuli and block 1. In block 2 AMTinnitus (Panel A) underwent loudness (loudness reduction by 30 dB and linear fade out) and temporal manipulations (duration of 6 minutes) resulting in 4 stimuli including the standard AMTinnitus stimulus from block 1.

to maintain vigilance and comfort of the participants. At the end of the study after block 2, the VAS for tinnitus loudness and tinnitus questionnaires were (again) filled in by the participants. Participants were then thanked for their participation and finally dismissed.

Data analysis

A repeated measures mixed model analysis of variance (ANOVA) was calculated with the factors time and condition as well as a random intercept per participant to assess the effect of temporary tinnitus suppression in the loudness growth paradigm. Post hoc tests of the ANOVA controlled for multiple comparisons contrasting the suppression profiles between the stimuli were performed using the Tukey method. Finally, paired two-tailed t-tests were used to compare tinnitus questionnaire scores and tinnitus loudness VAS before and after acoustic stimulation procedure. As the 3 variables subjected to the paired comparisons were considered within an independent analysis and not part of any primary outcome statistical model or search space, we refrained from a correction for multiple comparisons (e.g. bonferroni) for this secondary analysis. R statistic toolbox with the supplementary libraries 'nlme' and 'lsmeans' was used for all statistical calculations (R version 3.3.2; R Foundation for Statistical Computing, Austria).

Results

Tinnitus loudness growth after acoustic stimulation

The results of the ANOVA for the tinnitus loudness growth curves of all stimuli in block1 are shown in table 2.9 and respective corrected post-hoc contrasts in table 2.10. Notably, there was a significant effect of condition, time, and interaction condition*time on the tinnitus loudness. Mean tinnitus loudness suppression curves are plotted in figure 2.7.

Post hoc contrasts between each of the 7 stimuli elicited significant differences ($p<0.05$) for AMMusic vs. AMTinnitus ($t(27)=4.42$, $p<0.0001$), Pink noise vs. AMTinnitus ($t(27)=4.22$, $p=0.001$), AMLow vs. AMTinnitus ($t(27)=3.70$, $p=0.004$), AMFM vs. AMMusic ($t(27)=-3.31$, $p=0.016$), and AMFM vs. Pink Noise ($t(27)=-3.12$, $p=0.031$), respectively. These results are indicative of a pattern of enhanced tinnitus suppression of AMTinnitus and AMFM compared to Pink Noise, AMMusic, and AMLow (except AMFM vs. AMLow with $t(27)=-2.60$, $p=0.127$).

To counteract possible effects of the stimulation sequence in block 1, we furthermore tested the data for order effects with no significant results for position ($F(1,1317)=0.05$, $p=0.832$), condition*position ($F(6,1317)=0.94$, $p=0.468$), time*position ($F(1,1317)=3.05$, $p=0.081$), and interaction condition*time*position ($F(6,1317)=0.70$, $p=0.646$).

	numDF[a]	denDF[b]	F-value	p-value
(Intercept)	1	1331	2845.28	<0.0001
Condition	6	1331	5.40	<0.0001
Time	1	1331	185.81	<0.0001
Condition:Time	6	1331	3.74	0.0011

Table 2.9: **Results of ANOVA block 1 (n=28)**. [a]numDF = degrees of freedom of numerator. [b]denDF= degrees of freedom of denominator.

Contrast	Estimate	t-value	p-value
AMFM - AMMusic	**-5.204**	**-3.31**	**0.016**
AMFM - AMPinknotch	-2.245	-1.43	0.786
AMFM - AMLow	-4.082	-2.60	0.127
AMFM - Pink noise	**-4.898**	**-3.12**	**0.031**
AMFM - AMTinnitus	1.735	1.11	0.927
AMFM - Tinnitus pure tone	-2.041	-1.30	0.852
AMMusic - AMPinknotch	2.959	1.88	0.491
AMMusic - AMLow	1.122	0.72	0.992
AMMusic - Pink noise	0.306	0.20	>0.999
AMMusic - AMTinnitus	**6.939**	**4.42**	**<.0001**
AMMusic - Tinnitus pure tone	3.163	2.01	0.406
AMPinknotch - AMLow	-1.837	-1.17	0.906
AMPinknotch - Pink noise	-2.653	-1.69	0.623
AMPinknotch - AMTinnitus	3.98	2.53	0.148
AMPinknotch - Tinnitus pure tone	0.204	0.13	>0.999
AMLow - PinkNoise	-0.816	-0.52	0.999
AMLow - AMTinnitus	**5.816**	**3.70**	**0.004**
AMLow - Tinnitus pure tone	2.041	1.30	0.852
Pink noise - AMTinnitus	**6.633**	**4.22**	**0.001**
Pink noise - Tinnitus pure tone	2.857	1.82	0.535
AMTinnitus - Tinnitus pure tone	-3.776	-2.40	0.198

Table 2.10: **Post hoc contrasts block 1 (n=28, df[a]=1331, Tukey-adjusted)**. [a] df = degrees of freedom.

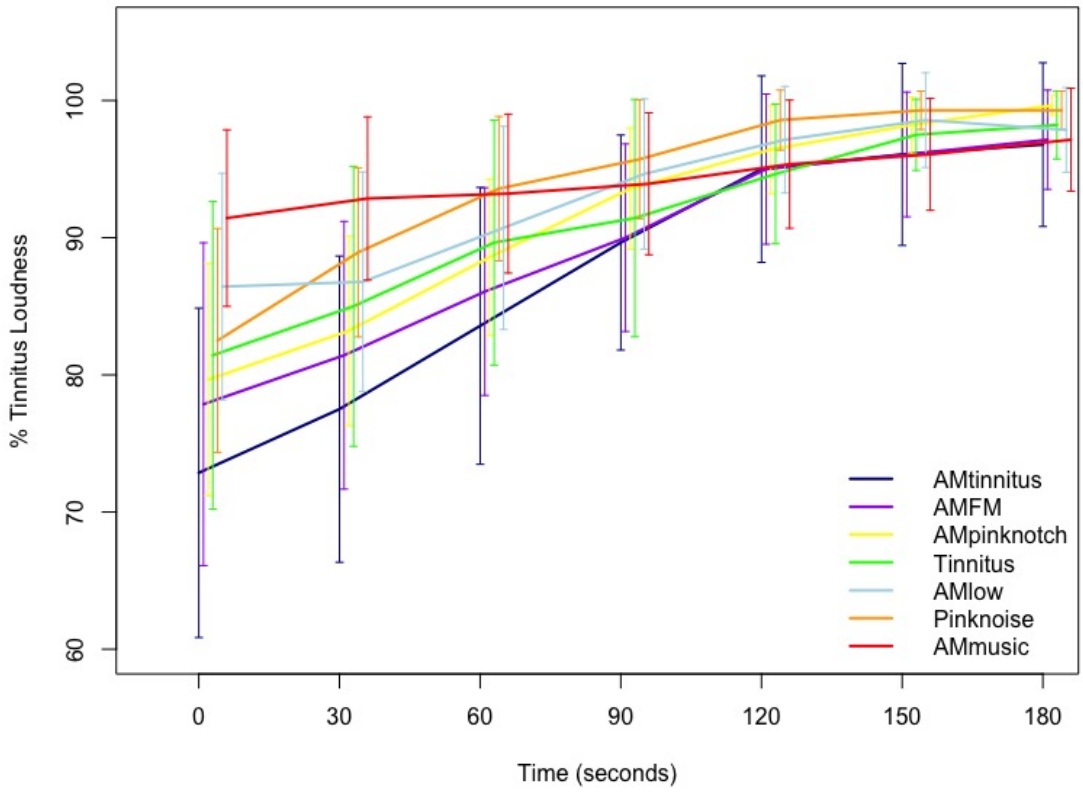

Figure 2.7: **Mean tinnitus loudness suppression after stimulus offset of all sound stimuli in block 1**. Confidence intervals at 95 % are plotted for each condition and time point. Notably, after 90-120 seconds tinnitus loudness suppression generally diminishes and curves of the different stimuli converge. Significant differences between stimuli (conditions) are listed in table 2.10.

For block 2, we report the results for tinnitus loudness growth of the manipulated variations of AMTinnitus (long (6 minutes of duration), fade, and reduced sound level) with the addition of the data of AMTinnitus of block 1 (standard) in table 2.11. Post hoc contrasts are indicated in table 2.12 and mean tinnitus loudness suppression curves are plotted in figure 2.8. Of special interest and according to our expectations, longer stimulation (long, 6 min) resulted in a larger suppression compared to stimulations reduced in sound level (fade vs. long: t(27)=3.88, p=0.00065; reduced sound level vs. long: t(27)=4.00, p=0.00041) but no significant differences with the AMTinnitus stimulation for 3 minutes from block 1 (long vs. standard: t(27)=-1.42, p=0.486). Furthermore, AMTinnitus elicited marginally increased suppression compared to the faded stimulus (fade vs. stan-

dard: t(27)=2.46, p=0.067, trend) and the stimulus with reduced sound level (reduced sound level vs. standard: t(27)=2.57, p=0.050). The comparison of the two stimuli with manipulated sound level resulted in no significant difference (fade vs. reduced sound level: t(27)=-0.12, p=0.999).

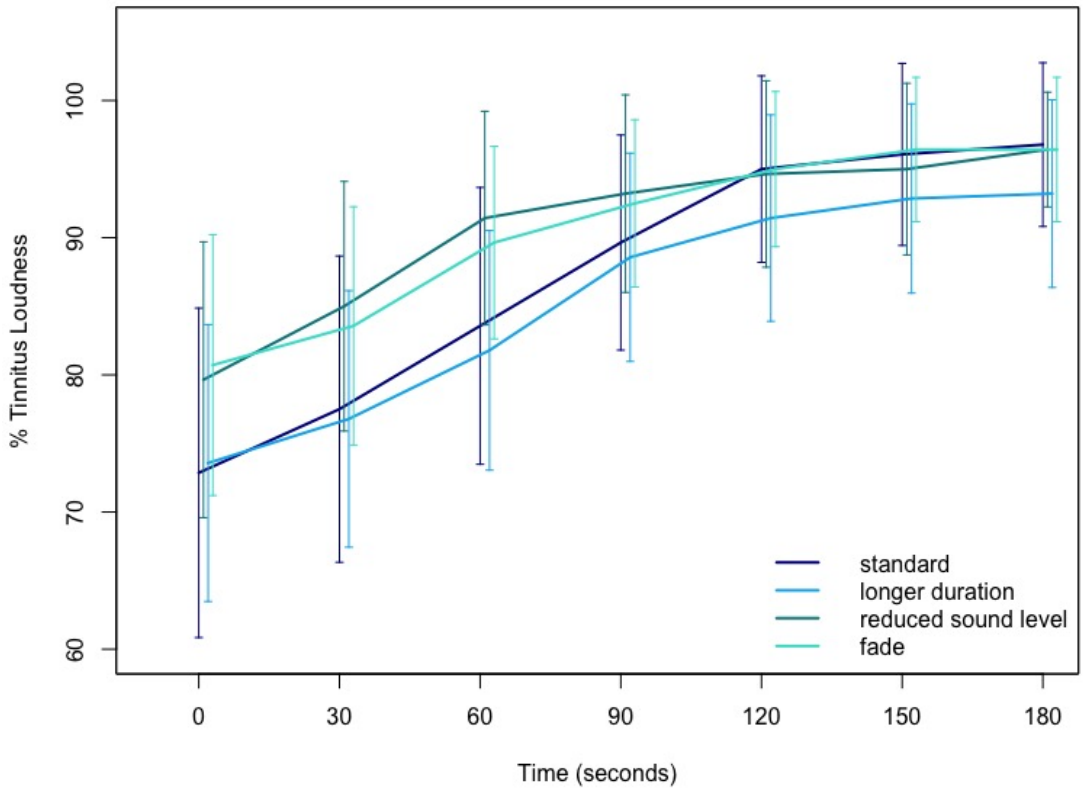

Figure 2.8: **Mean tinnitus loudness suppression after stimulus offset of AMTinnitus and its variations in block 2**. Confidence intervals at 95 % are plotted for each condition and time point. Standard and longer duration of the stimulus are colored in blue whereas stimuli with reduced sound level or fade out are colored in green. Significant differences between stimuli (conditions) are listed in table 2.12.

Responder patterns and overall feasibility

The evaluation of the matched tinnitus pitch resulted in a mean of 4.0 (SD=0.55, with 5 indicating perfect fit) highlighting the reasonable quality of the matching procedure. The response criterion for temporary tinnitus suppression was set to any suppression per stimuli (here at t0, right after the offset of the auditory stimulation) as similarly done before

	numDF[a]	denDF[b]	F-value	p-value
(Intercept)	1	749	746.20	<0.0001
Condition	3	749	7.62	0.0001
Time	1	749	201.14	<0.0001
Condition:Time	3	749	2.70	0.0443

Table 2.11: **Results of ANOVA for AMTinnitus in block 2 (n=28)**. [a]numDF = degrees of freedom of numerator. [b]denDF= degrees of freedom of denominator.

Contrast	Estimate	t-value	p-value
fade - reduced sound level	-0.153	-0.12	0.999
fade - long	**5.153**	**3.88**	**0.00065**
fade - standard	3.265	2.46	0.067
reduced sound level - long	**5.306**	**4.00**	**0.00041**
reduced sound level - standard	**3.418**	**2.57**	**0.050**
long - standard	-1.887	-1.42	0.486

Table 2.12: **Post hoc contrasts block 2 (n=28, AMTinnitus, df=749[a], Tukey-adjusted)**. [a]df=degrees of freedom.

(Reavis et al., 2012). Applying this criterion, the following descriptive responder pattern emerges: In the AMTinnitus condition 19 out of 28 participants indicated a suppression at t0, in AMPinknotch 19/28, in AMFM 16/28, in Pink noise 16/28, in AMLow 13/28, in Tinnitus pure tone 13/28, and in AMMusic 8/20.

Differences in tinnitus loudness (VAS) and total scores of standardized questionnaires (TQ and THI) comparing respective assessments before and after experimental procedures are listed in table 2.13 and summarized in the following. Tinnitus loudness (VAS) was significantly reduced after experimental procedures compared to the baseline assessment ($t(27)=2.774$, $p=0.01$). Furthermore, TQ and THI scores measuring tinnitus-related distress were also both lower after the experiment. While TQ scores are below the p-value threshold of $p=0.05$, we can only report a trend for the THI (TQ: $t(27)=2.062$, $p=0.049$; THI: $t(27)=1.922$, $p=0.065$). It has to be noted though, that the effects reported here are based on the possible influence of all amplitude modulated sounds as well as unmodulated 'control' sounds and this secondary analysis serves safety and feasibility purposes.

Measure	Mean score pre	SD[a] pre	Mean score post	SD post	df	t-value	p-value
VAS loudness (mm)	54.46	17.39	48.25	17.48	27	2.774	0.01
TQ total score (0-84)	38.36	13.09	35.07	14.78	27	2.062	0.049
THI total score (0-100)	42.25	16.3	38.29	16.95	27	1.922	0.065

Table 2.13: **Differences in tinnitus loudness and questionnaire scores before and after experimental procedures**. [a]SD = Standard Deviation.

Discussion

Acoustic stimulation or sound therapy is proposed as a main treatment option for chronic subjective tinnitus (Langguth et al., 2013). Numerous approaches for acoustic stimulation exist, be it in experimental studies (e.g. (Roberts et al., 2006; Reavis et al., 2012; Hoare et al., 2014)), longitudinal clinical trials (e.g. (Adamchic et al., 2014; Okamoto et al., 2010)), fitted hearing aids or sound players (e.g. (Vernon and Meikle, 2003)), mobile apps or webpages, and various user-driven self-administered forms. As of yet, there is neither an established general-purpose acoustic stimulation to abolish or reduce tinnitus nor a working strategy for subtypization of responder profiles. To further probe the field of acoustic stimulations for tinnitus therapy, the purpose of this exploratory study was to compare 10 Hz AM sounds (pure tones, noise, music and FM sounds) and unmodulated sounds (pure tone, noise) regarding their temporary suppression of tinnitus loudness in participants with tonal tinnitus.

First we found that all sounds elicit a short-term suppression of tinnitus loudness (seconds to minutes) with strongest suppression right after stimulus offset . Adding to this, feasibility of the overall procedure could be confirmed as scores of both tinnitus questionnaires as well as the VAS for tinnitus loudness were lower after the experiment . Furthermore, no adverse events or persisting increase in tinnitus loudness or distress during and after the experimental procedure were noted. Second, akin to the findings of Reavis et al. (2012), while not directly comparable (due to higher presentation loudness, frequency ranges instead of matched tinnitus pitch and white noise instead of pink noise in our study), we found that AMTinnitus and AMFM produced a significantly stronger tinnitus loudness suppression than noise . Furthermore, both AMTinnitus and AMFM produced superior suppression than AMMusic condition with the amplitude modulated notch filter . Finally, AMTinnitus resulted in a clearly more pronounced suppression than

AMLow . Taken together, these results imply that AM sounds, especially in or around the tinnitus frequency (i.e. AMTinnitus and AMFM, (Schaette et al., 2010)), may produce larger suppression than unmodulated sounds. Yet, the direct contrast between AMTinnitus and Tinnitus pure tone did not result in a significant difference, but the direction and the size of the statistical values may point to a significant contrast in future studies (see figure 2.7 and table 2.10). Possible cumulative effects of tinnitus suppression over the entire acoustic stimulation procedure in block 1 can be largely ruled out as there were no order effects . Third, with the manipulations of the AMTinnitus stimulus in block 2 either increasing the stimulus duration to 6 minutes, or reducing either overall sound level (30 dB), or fading of the stimulus in the last minute, we could partly show that these manipulations led to an altered tinnitus suppression: Standard AMTinnitus produced significantly more tinnitus suppression than both of the sound level-manipulated variations according to our expectations (i.e. reduced sound level, see figure 2.8 and table 2.12), yet the longer version of the very same stimulus failed to show increased overall tinnitus suppression . However, comparing the loudness growth curves of the standard AMTinnitus with the version longer in duration, there may be a difference in suppression depth from 90 seconds onwards after stimulation offset. While the initial suppression at 0 seconds seems to be in similar range in both stimuli, the longer version may sustain the suppression for a longer time as reflected in the flatter curve. This effect could be topic of possible future studies where stimulation duration undergoes respective manipulation.

Looking at the AMFM stimulus we noticed both a good suppression potential second to AMTinnitus and a promising tolerance as participants clearly preferred AMFM over all other stimuli for block 2 (10/28 chose AMFM out of the 7 alternative options). On the other hand, it is challenging to interpret these results given the lack of a direct control sound (i.e. 10 Hz FM without AM). Finally, the sounds with amplitude modulated notch filter (AMPinknotch and AMMusic) were designed to test possible short-term suppression effects of the established long-term sound therapy with notch-filtered music (Pantev et al., 2012). AMMusic clearly exhibited the least overall suppression probably due to missing energy of the sounds in and around the filtered frequency range inherent to the presented songs, as music is both spectrally and temporally highly variable in amplitude (cf. figure 2.6, panel E). To a lesser degree, this is also true of (pink) noise so that both of the notch-filtered AM sounds are certainly not straight-forwardly comparable in acoustic

morphology and putative suppression effects to the pure tone sounds. Furthermore, give the tonal nature of the tinnitus in participants, this result certainly was expectable. All in all, the weaker suppression effect of these filter gain modulated sounds may be due to missing energy in the critical frequency bands of the notch filter which is not surprising given the long-term application and and its putatively induced reversal of maladaptive map plasticity through residual inhibition (Pantev et al., 2012; Tass et al., 2012).

Generally, between 90 and 120 seconds after stimulus offset, or even earlier in some stimuli (i.e. AMMusic, Pink noise, AMPinknotch, AMLow), tinnitus loudness reaches 90% of the baseline loudness and tends to reach 100% after 180 seconds, which equals the stimulation duration. A similar pattern was observed by Reavis et al. (2012) in representative, individual suppression profiles while group statistics is not performed in a comparable manner to our study. First, we did not focus on responders for statistical analyses like the previous study as all subjects, conditions and time points were included in our study. Second, no transformation on the variables or other adjustments to the raw data were performed. Yet, given the various differences in the study design of (Reavis et al., 2012), namely measuring suppression during acoustic stimulation, having reference tones in and after the stimulation and applying a loudness regime slightly below matched tinnitus, results are still deemed comparable and we may substantiate the former findings that AM (and partly FM) sounds elicit better tinnitus suppression than traditional maskers (i.e. unmodulated white noise and pure tones).

Limitations

In the following we would like to consider some issues, which may be regarded as shortcomings of our study, while not being detrimental given the exploratory scope of this study. First, looking at the sound stimuli, unlike Reavis et al. (2012) we did not use white noise as (control) masking sounds, which may limit the interpretation of especially the contrast to AMTinnitus, as in white noise there is more sound energy in the high frequency bands where tinnitus usually manifests. Besides, there also was no direct, unmodulated control sound to AMLow and noise was not amplitude modulated over the entire audible frequency range. Future studies should therefore define respective a priori contrasts with only a single or few parameters manipulated in the stimuli to ensure optimal comparability. Second, sound presentation may be updated with consideration of

tinnitus laterality (contra- vs. ipsi- vs. bilateral presentation) (Feldmann, 1971) and with related adjustments for asymmetrical hearing loss (Roberts et al., 2008), loudness weighting reconsidered (i.e. application of more detailed loudness contour curves (ISO 226) to the stimuli instead of dbA weighting), and finally matching sounds alongside the active stimuli to evaluate loudness growth independent from tinnitus (Reavis et al., 2012). Third, to both identify and analyze tinnitus subgroups as well as responder profiles, it would advantageous to include further questionnaires to probe comorbidities and (general) quality of life (Langguth et al., 2007) and, more importantly, questionnaires elucidating personal profiles, like the NEO-PI-R (Costa and McCrae, 2008), possibly related to tolerance and acceptance of sound therapy in tinnitus. Fourth, given the behavioral nature of the current study, both neurophysiological models for cortical and subcortical responses to these stimuli and possible beneficial effects for tinnitus have to be specifically tested in fitting paradigms in future studies. Finally, in block 2, we could not test for order effects because the conditions with the stimuli chosen by the participants were deliberately left out in the analysis of the data. Given the inexistence of such order effects in block 1 and identical randomization strategies used in both blocks, we do not expect an order effect in the trimmed analysis of block 2.

Conclusion and outlook

Given the results of the present study in the context of previous findings, we conclude (and partly replicate) that amplitude modulated sounds with various carrier sounds in and around tinnitus frequency are feasible for short-term tinnitus suppression. With a modulation rate of 10 Hz in the EEG α band, we expect indirect neuromodulation and normalization of the endogeneous (also: individual) α rhythm which has been shown to be reduced in patients with tinnitus. Exact mechanisms of this auditory entrainment should therefore be investigated by means of respective neurophysiological methods (MEG/EEG) to test if and how auditory entrainment and possibly related tinnitus suppression is reflected by neural oscillations. Beyond that, longitudinal studies in real life should be performed to evaluate the envisioned long-term goal of this approach, namely to develop individually-customized mobile tinnitus sound therapies with aesthetically appealing sounds.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

PN and WS: substantial contribution to the design of the study, data analysis, drafted and revised the manuscript. JM: substantial contribution to the design of the study and data acquisition. MM: substantial contribution to the discussion of the approach and paradigm. MS and BL: drafted and revised the manuscript.

Funding

This research was supported by by the University Research Priority Program 'Dynamics of Healthy Aging' of the University of Zurich, the 'Fonds zur Förderung des akademischen Nachwuchses' (FAN) des 'Zürcher Universitätsvereins' (ZUNIV) and by TINNET - COST Action BM1306 'Better Understanding the Heterogeneity of Tinnitus to Improve and Develop New Treatments'.

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2.3 Sinusoidal amplitude modulated tones elicit stronger residual tinnitus suppression than pure tones

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A similar version of this manuscript has been submitted for publication to *Scientific Reports*.

Abstract

Acoustic stimulation has been proposed as one of the major avenues for tinnitus relief. Recent studies have compared the tinnitus suppression, or residual inhibition, between amplitude- and frequency modulated (AM/FM) sounds and established sounds like noise or pure tones (PT). Results of these studies showed stronger tinnitus suppression for the modulated sounds in contrast to unmodulated sounds. Yet, the observed effects were not very convincing possibly due to the explorative nature of the studies and related heterogeneous research designs. The aim of the current study is therefore to further advance this line of research by contrasting tinnitus suppression profiles of AM and PT sounds at the matched tinnitus frequency (10 and 40 Hz AM vs. PT). Participants with chronic, tonal and bilateral tinnitus (n=29) underwent comprehensive psychometric, audiometric as well as acoustic stimulation procedures. Results demonstrate better tinnitus suppression for the AM compared to the PT sounds, especially for the 10 Hz modulation and presentation levels of 60 dB SL. Furthermore the AM stimulus class was better tolerated as elicited by measures of valence and arousal. We conclude that, given the efficacy, tolerability and simplicity of use of the AM stimulus class, these sounds may qualify as a future sound therapy for tinnitus.

Introduction

Subjective tinnitus is defined as the perception of a phantom sound in the absence of any objective physical source (Eggermont and Roberts, 2004) and is defined as chronic after continuous presence for 6 months (Mazurek et al., 2010). Chronic subjective tinnitus is highly prevalent with 10 - 15 % of the population reporting continuous tinnitus perception and about 1 - 2 % suffering immensely from the condition (Langguth et al., 2013). The phenomenon is continuously gaining relevance as it coincides with a steadily

aging demographic (Hoffman and Reed, 2004) and concomitant age-related hearing loss (presbycusis) (Ferreira et al., 2009), with noisy occupational or leisure time environments (Shargorodsky et al., 2010; Sanchez et al., 2016), and with stress (Mazurek et al., 2012). Moreover, tinnitus not only related to altered auditory functions like speech perception (Moon et al., 2015; Ivansic et al., 2017), sound source localization (Hyvärinen et al., 2016) and auditory attention (Cuny et al., 2004) but also emotional attentional processes (Trevis et al., 2016), but also to depression and/or anxiety (Langguth et al., 2011), insomnia (Croenlein et al., 2016) and lowered quality of life (Weidt et al., 2016; Nondahl et al., 2007).

In most cases, the perception of the phantom sound seems to develop after either objective (Eggermont and Roberts, 2004; Mazurek et al., 2010; Schaette and Kempter, 2006) or hidden hearing loss (Schaette and McAlpine, 2011; Weisz et al., 2006; Adjamian et al., 2012), where loss of cochlear hair cells or other peripheral alterations leads to maladaptive plasticity in the auditory pathway and brain. Beyond the established concept that (a form of) hearing loss seems to always co-occur with tinnitus, tinnitus may also develop with stress or various pathologies of peripheral and central organs (Langguth et al., 2013). Models of tinnitus generation and maintenance are still being debated (Sedley et al., 2016) and limited by an underlying inherent heterogeneity of the disorder (Landgrebe et al., 2012). Yet, consensus arose that both the peripheral auditory system as well as differential brain networks are involved and correlate with differential aspects of tinnitus (Elgoyhen et al., 2015; De Ridder et al., 2014; Schlee et al., 2009b; Eggermont and Roberts, 2004; Jastreboff, 1990; Adjamian et al., 2009; De Ridder et al., 2011).

Up to today, there is no generally applicable cure for this often tantalizing phantom sound perception. Established interventions aim at alleviating the tinnitus sound or accompanying symptoms (Baguley et al., 2013). Within a consensus clinical management framework (Langguth et al., 2013), three avenues of symptom-oriented interventions are suggested. First and ideally accompanying other options (Baguley et al., 2013), cognitive behavioral therapy (CBT) is suggested to establish coping strategies (Cima et al., 2012). A further option are differential approaches of neuromodulation -and stimulation (Hoare et al., 2016; Soleimani et al., 2016) with concurrently increased efficacy with multi-site montages (Lehner et al., 2016), individual protocols (Kreuzer et al., 2017) and possibly combined approaches (Teismann et al., 2014; Shekhawat et al., 2015). Finally, auditory

stimulation and therapy were traditionally studied and evolved to exert maximal efficacy in suppressing tinnitus in sound therapies (Feldmann, 1971; Vernon, 1977; Hazell and Wood, 2009; Henry et al., 2004b; Watanabe et al., 1997). Also here, recent technical advances and neuroscientific backing could spawn some promising approaches of auditory retraining mirrored by (related) reversal of maladaptive neural plasticity caused by tinnitus (Okamoto et al., 2010; Tass et al., 2012; Adamchic et al., 2017; Stein et al., 2015). Yet, whereas masking for example alongside counseling in tinnitus management has proven efficacy and may be clinically implemented (Baguley et al., 2013), there is still debate about efficacy of aforementioned retraining approaches (Wegger et al., 2017)

The current study joins the branch of auditory stimulation in tinnitus with a focus on residual inhibition (RI) or tinnitus suppression effects with patterned (here: amplitude modulated (AM)) sounds. Recent studies have demonstrated more pronounced tinnitus suppression after stimulation with amplitude and/or frequency modulated sounds compared to unmodulated sounds and noise (sounds in different frequency bins modulated with 40 Hz (Reavis et al., 2012), wide array of sounds including matched tinnitus PT modulated with 10 Hz (Neff et al., 2017)). This effect is primarily observed when comparing modulated with PT sounds in or around the tinnitus frequency (Schaette et al., 2010) while its exact mechanisms of action remain unclear. Concretely, it is not known how modulated sounds produce stronger and longer tinnitus suppression or residual inhibition (RI, (Roberts, 2007)) than constant noise or PT sounds. This is partly explicable by the fact that in classical masking and RI only unmodulated sounds have been used (e.g., (Terry et al., 1983; Roberts et al., 2006, 2008)). Alternatively or concomittantly, neural entrainment effects may account for an normalization of tinnitus-specific neural oscillations as theorized in our previous article (Neff et al., 2017) and in comparable disorders (Ecsy et al., 2017). Neural entrainment describes the phenomenom of synchronization of endogenous neural oscillations to patterned or rhythmic external stimuli (here: auditory (Picton et al., 2003; Draganova et al., 2008)). Furthermore, changes in neurophysiology (Kaltenbach and Godfrey, 2008) or neurochemistry (Sedley et al., 2015) throughout the auditory pathway and the brain may also play a role but have to be specifically tested and modelled with the modulated stimulus class. Yet, given the multitude of possible mechanisms of action, the ongoing research on causes and mechanisms and the underlying problem of heterogeneity of tinnitus, limited methods, and the gap between human

and basic animal research, it is difficult to propose an all-encompassing model of the mechanism of action of AM stimulation. Beyond that, data is scarce and largely absent in the case of prolonged (i.e. long time interventional) stimulation with the modulated stimulus class. Therefore, it is deemed necessary to proceed in small steps and currently focus on the immediate subjective effects towards later application in clinical studies and possible interventions. As in other acoustic stimulation approaches (Tass et al., 2012; Okamoto et al., 2010; Henry et al., 2015) the approach of modulated sounds presented here is deemed highly individually tailorable and applicable in mobile interventions.

Building on these observations including the efficacy of 10 Hz AM sounds at the matched tinnitus frequency in our former study (Neff et al., 2017) and the effects of 40 Hz AM and FM sounds found by Reavis and colleagues (Reavis et al., 2012), the aim of this study is to specifically compare AM with PT sounds at the matched tinnitus frequency. Concretely, we hypothesize that AM sounds (10 and 40 Hz modulation) at the tinnitus frequency may elicit better short-term tinnitus suppression than their unmodulated PT pendants.

Furthermore, we wanted to test if and how different sound levels during acoustic stimulation may influence tinnitus suppression by presenting the stimuli at sensation level (SL) plus 60 dB as in our former study compared to presentation slightly above individual's minimum masking level (MML). While we expect better tinnitus suppression for the SL stimuli due to the higher presentation loudness compared to the MML stimuli, we still hypothesize that the effect of better suppression of AM compared to unmodulated sound will become evident in both loudness regimes. Aiming at possible future acoustic interventions for tinnitus relief, subjective evaluation and tolerability of the stimuli is deemed as critical so and was assessed by means of pictorial scales (manikins) of valence and arousal (Bradley and Lang, 1994). Here, we expect better tolerability (reflected by higher valence and lower arousal scores) for the AM compared to the PT sounds. To the best of our knowledge this represents the first study of this kind in the field and implications may be critical for developing future sound therapies for tinnitus.

Methods

Methods, procedures and sample size of the study are directly comparable to our former study with some changes in the tinnitus matching equipment and protocol (Neff et al.,

2017). Numeric participant characteristics, tinnitus parameters and tinnitus matching results are listed in table 2.15.

Participants

29 patients (9 female, between ages 18 and 75) with chronic tonal tinnitus (>12 months since tinnitus onset) from the Interdisciplinary Tinnitus Clinic of Regensburg were included in this study. Patients with a history or presence of any severe and relevant somatic, neurological, or mental disorders were excluded. Further exclusion criteria were ongoing intake of any psychotropic medication or substance and the participation in other tinnitus studies or treatments. The study was approved by the local ethics committee (16-101-0061). After a comprehensive explanation of the procedures, risks and benefits all participants gave written informed consent.

Psychometry

Upon the actual experiment, participants filled out an online questionnaire comprising the Tinnitus Sample Case History Questionnaire for clinical and demographic data (Langguth et al., 2007), the Tinnitus Questionnaire (Goebel and Hiller, 1994), the German adaption of the Tinnitus Handicap Inventory (Newman et al., 1996), and the short version of the Hyperacousis Questionnaire (mini-HQ9 (Berthold-Scholz, 2013)).

Audiometry

Hearing thresholds were measured in the frequency range from 125 Hz to 8 kHz in octave steps with semi-octave steps between 0.5 and 1 (i.e. 0.75 kHz), 1 and 2 (i.e. 1.5 kHz), 2 and 4 (i.e. 3 kHz), and 4 and 8 kHz (i.e. 6 kHz), respectively (Madsen Midimate 622D; GN Otometrics, Denmark). Identical headphones with quasi flat linear frequency response were used for audiometry, subsequent tinnitus matching and the actual acoustic stimulation procedure (Sennheiser HDA 2000; Sennheiser, Germany).

Tinnitus matching

Tinnitus matching was performed applying a mode of adjustment approach (MOA, (Henry et al., 2004a)) with a custom-tailored MAX program (MAX 7; Cycling'74, USA) and a modular hardware controller (Palette Expert Kit; Palette; Canada). We adhered to a modified Tinnitus Tester procedurality ((Roberts et al., 2008) without tinnitus likeliness ratings,

tests for RI, loudness matching of 1 kHz reference tones but with the inclusion of an octave confusion test at the end of the procedure) while accustoming the participants to the matching equipment. Main parameters of interest assessed by the matching procedure were tinnitus loudness (in dB), tinnitus side (on a continuum between 0 (= left ear) to 127 (= right ear) with the value of 63 representing equally distributed bilateral tinnitus) and tinnitus frequency (in Hz). During the actual matching procedure participants self-reliantly adjusted all the parameters with no need to check with the study personnel or the computer screen (tinnitus parameters were indicated on the controller upon touching of the respective control units). Finally participants were given the opportunity to rate the correspondence between matched sound and their tinnitus as well as the general usability of the matching equipment on a scale ranging from 1-10. The time of the self-reliant matching procedure was assessed by the study personnel and the matching procedure was repeated after acoustic stimulation described in the next paragraph.

Acoustic stimulation

5 amplitude modulated sounds (10/40 Hz modulation rates at 60 dB SL and MML of presentation loudness, and an inaudible single 10 Hz stimulus 6dB below SL) and 2 unmodulated sounds (PTs at 60 dB SL and MML) were prepared in MATLAB (Matlab R2015a; Mathworks, USA) with the matched tinnitus pitch acting as the frequency of the PT carrier sounds. In the remainder of the manuscript the stimuli are termed as follows (table 2.14). AM1060 refers to the AM sound modulated with 10 Hz at 60 dB SL, AM10MML to the 10 Hz AM sound at 6 dB above MML, AM4060 to the 40 Hz AM sound at 60 dB SL, AM40MML to the 40 Hz AM sound at 6 dB above MML, P60 to the PT at 60 dB SL, PMML to the PT at 6 dB above MML, and finally AM10U to the undetectable 10 Hz AM sound 6 dB below SL.

Stimulation level / Modulation rate (Hz)	60 dB SL	MML + 6 dB	SL - 6dB
0	P60	PMML	-
10	AM1060	AM10MML	AM10U
40	AM4060	AM40MML	-

Table 2.14: **Overview and nomenclature of the acoustic stimuli**.

The sum total of 7 acoustic stimuli with 3 min of duration each was produced for each participant individually. Details about the stimulus creation are indicated in paragraph 2.3 and figure 1 of our previous publication (stimuli in the current study correspond to the 'AMTinnitus' stimulus in the former study) (Neff et al., 2017).

Stimuli were presented at either 60 dB SL, 6 dB above MML or 6 dB below SL in the case of AM10U. 80 dBA was the upper limit for the sound level of all stimuli, which was checked with an SPL meter (NTi Audio XL2; NTi Audio, Lichtenstein). Participants were reminded to interrupt the procedure whenever a sound was deemed uncomfortable. No particular instruction was given to focus their attention on either the sound or tinnitus. Presentation sequence of the 7 stimuli was randomized for each participant. Participants were instructed to rate the loudness of their tinnitus in percent, compared to the pre-stimulation loudness, after each stimulation at time points 0, 30, 60, 90, 120, 150, and 180 seconds (Neff et al., 2017; Reavis et al., 2012). Furthermore participants rated all stimuli in valence and arousal (Bradley and Lang, 1994). At the end of the stimulation procedure participants again performed the tinnitus matching task and were finally dismissed.

Data analysis

R (R version 3.3.3; R Foundation for Statistical Computing, Austria) was used to calculate statistics including descriptives, pearson correlations and paired samples t-test to test the differences in evaluation of the stimuli. To investigate the main research question, namely the difference between modulated and unmodulated sounds at the tinnitus frequency, linear mixed effect models were computed with the nlme package (https://cran.r-project.org/web/packages/nlme/) with fixed effects for condition (i.e. different acoustic stimuli), random effects for time and subjects, and an added term for position (within the order of the presented 7 stimuli) as a covariate, both modelled linearly and quadratically for optimal model fit. A priori contrasts of interest were defined between AM and PT conditions for both stimulation level regimes (i.e. 10 and 40 Hz AM vs. PT sounds at 60 dB SL and MML). The model was fitted using the maximum likelihood (ML) method unbiased for the fixed effects and appropriate for the given sample size. For the contrasts, both corrected (Bonferoni adjustment for the number of contrasts) and uncorrected results are reported side by side in the results section. Given prior work (Reavis et al., 2012; Neff et al., 2017) and resulting hypotheses, we expect larger suppression for modulated

sounds and therefore adjusted the final p-values for one tail. For the exploratory analysis of valence and arousal related to the stimuli, two-tailed tests were used given the lack of a (directed) hypothesis.

Results

Participant characteristics and audiometry

Participants characteristics, questionnaire scores and main tinnitus matching parameters are listed in table 2.15. Hearing thresholds did not differ between the two ears (left side: mean = 21.21, SD = 9.54; right side: mean = 20.96, SD = 11.03; t (28) = 0.36, p = 0.722).

According to the participation prerequisite of bilateral tonal tinnitus, 11 participants indicated their tinnitus location in both ears, 3 inside the head, 6 in both ears stronger in the left one, 4 in both ears stronger in the right one, 1 in the left ear, and 4 in the right ear.

Tinnitus matching

Results of the matching procedure before acoustic stimulation are listed in table 2.15. Participants ratings of the matched sound and the matching procedure were high (matched sound: m = 8.66, SD = 0.936. matching procedure: m = 8.62, SD = 1.237. (range 1-10)) further building confidence towards validity of the method and possibly critically contributing to the efficacy of the individually tailored sounds. Average time spent for matching was 382 seconds (SD = 207).

Despite some indications of transient alterations of the quality of the tinnitus percept seconds after stimulation, no persisting alterations of the percept after single stimulation or the entire experiment were observed. Moreover, there were no significant differences of matching parameters, namely tinnitus frequency, loudness, and side (t(max) = -0.644, p(min) = 0.525) between the matching procedures before and after the actual stimulation. This furthermore enhances confidence in the applied matching method reflected by high correlations between matching parameters of interest (tinnitus frequency: r = .826, p $<$0.001. loudness: r = .833, p $<$0.001. side: r = .937, p $<$0.001).

Acoustic stimulation

The mean tinnitus loudness suppression profile over time after stimulus offset can be seen in figure 2.9. Notably, as observed in our previous study (Neff et al., 2017), tinnitus

	Mean	SD	Median	Minimum	Maximum
Age (years)	54.72	11.26	57.00	22	73
Tinnitus duration (months)	168.97	113.92	132.00	16	420
Hearing loss (both ears, dB)	21.08	10.14	19.09	3	44
Sensation level near tinnitus frequency (both ears, dB)[a]	33.45	18.67	30.00	0	70
TQ total score (0-84)	36.83	17.22	40.00	10	63
THI total score (0-100)	53.10	11.26	53.00	33	71
Mini-HQ9 (0-27)	12.38	5.39	11.00	4	24
Tinnitus awareness (%)	66.00	25.74	70.00	20	100
Tinnitus loudness (%)	59.83	21.90	60.00	20	100
VAS loudness (0-100)	50.90	2.59	50.90	1	90
MML (dB)	60.28	18.05	58.00	29	80
Tinnitus loudness (matching, dB)	57.72	15.38	56.61	19	80
Tinnitus frequency (matching, Hz)	4040.66	2122.25	3530.00	298	10965
Tinnitus side (matching, 0-127, left ear = 0/center = 63/right ear = 127)	66.66	35.53	63.00	0	127

Table 2.15: **Participant characteristics and tinnitus parameters (n=29)**. [a] = Nearest frequency in pure tone audiometry to the matched tinnitus frequency. TQ = Tinnitus Questionnaire (Goebel and Hiller, 1994). THI = Tinnitus Handicap Inventory (Newman et al., 1996). Mini-HQ9 = Mini Hyperacousis Inventory (German, (Berthold-Scholz, 2013)). VAS = visual analog scale. MML = minimum masking level.

	numDF	F-value	p-value
Intercept	1	8452.589	<0.001
condition	6	22.495	<0.001
time	1	7.962	0.005
poly(position, 2)	2	16.155	<0.001
condition : time	6	4.721	<0.001

Table 2.16: **Anova of the main linear mixed effects model**. Degrees of freedom = 1377. Notably, unlike in our previous study, an effect for position (order effect) is discernible while we observe significant effects for all main effects and the interaction condition*time. Poly = polynomial term (2 = quadratic).

suppression is strongest 0 seconds after stimulus offset for all stimuli except AM10U and converges towards pre-stimulation loudness after 90 seconds towards 180 seconds. AM sounds at 60 dB SL exerted the strongest suppression (AM1060 and AM4060) followed by their variations at MML and the PT at 60 dB SL. Finally, PMML and AM10U produced only slight or no suppression, respectively. The results of the omnibus anova are listed in table 2.16 and, in contrast to our previous study, indicative of a significant effect for position within the presentation order of the stimuli.

Within the linear mixed effects model, the contrasts of interest between AM1060/AM4060 and P60, and AM10MML/AM40MML and PMML, respectively, were significant for AM1060 vs. P60 but not for AM4060 vs. P60. This finding substantiates the observed trend in our previous paper, partly confirms our hypotheses and is in line with similar observations of Reavis and colleagues that PTs produce less tinnitus suppression than their AM pendants. On the other hand, looking at stimulation levels near the tinnitus' actual loudness (slightly below tinnitus loudness as in (Reavis et al., 2012) and 6 dB above MML in our study) only trends at the uncorrected level can be observed for both 10 and 40 Hz stimuli.

As we identified an effect for position in the main model, we evaluated this position effect in an ancillary model seen in table 2.18 to probe possible influences on the interpretation of the main results. The difference between AM4060 and P60 seems to grow as a function of position so that at positions late in the experiment the difference is contaminated by the influence of position. In consequence, and in contrast to the prima facie impression of similar suppression curves of AM1060 and AM4060 in figure 2.9, this may explain the null-finding of the contrasts AM4060 vs. P60 in the main model with position

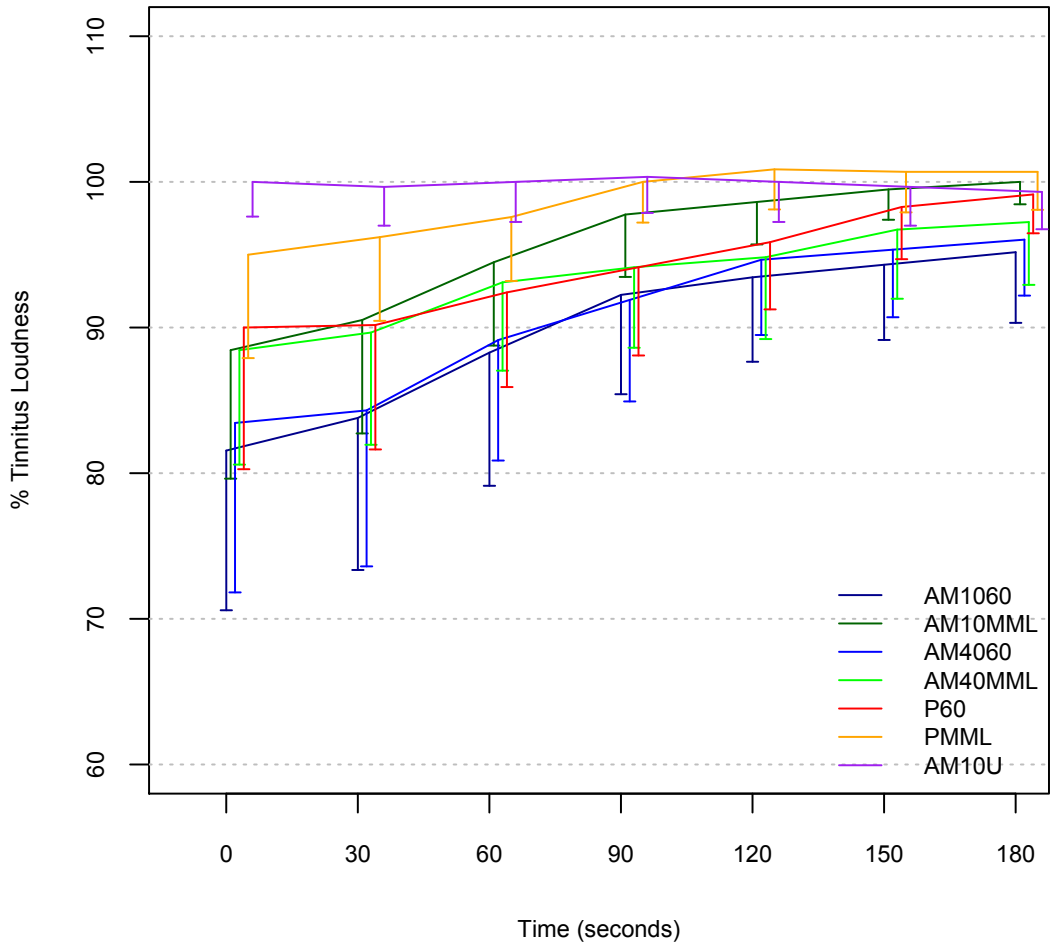

Figure 2.9: **Mean tinnitus suppression after stimulus offset for all stimuli**. Brackets indicating 95% confidence interval for each condition, respectively. Generally, AM sounds tend to elicit stronger and more sustained tinnitus suppression compared to PTs except the AM10U condition where the sound was presented 6dB below sensation level. Main contrasts of interest between AM and PT conditions for both stimulation levels show more tinnitus suppression for AM1060 vs. P60 ($t = 2.417$, p (bonf.) $= 0.032$) and uncorrected trends ($p < 0.1$) for AM10MML vs. PMML ($t = 1.914$, $p = 0.056$) and AM40MML vs. PMML($t = 1.781$, $p = 0.075$), table 2.17.

	Value	SE	t-value	p-value	p-value (bonf.)
Intercept	89.955	3.469	25.929	<0.001	
AM1060 - P60	2.840	1.175	2.417	0.016	0.032
AM4060 - P60	1.308	1.173	1.116	0.265	0.529
AM10MML - PMML	2.248	1.175	1.914	0.056	0.112
AM40MML - PMML	2.089	1.173	1.781	0.075	0.150

Table 2.17: **Results of the contrasts of interest in the main linear mixed effects model**. Degrees of freedom = 1377. Main contrasts of interest between AM and PT conditions for both stimulation levels show more tinnitus suppression for AM1060 vs. P60 (t = 2.417, p (bonf.) = 0.032) and uncorrected trends (p <0.1) for AM10MML vs. PMML (t = 1.914, p = 0.056) and AM40MML vs. PMML(t = 1.781, p = 0.075). SE = Standard Error.

as a covariate. Therefore we cautiously interpret this finding that 10 Hz AM sounds may elicit larger tinnitus suppression than 40 Hz AM compared to PT sounds.

Stimulus evaluation

Valence and arousal scores for the whole set of stimuli are plotted in figure 2.10 and contrasts of interest listed in table 2.19. Of particular interest and according to our hypotheses, valence was rated significantly higher for AM1060 vs. P60 whereas only trends were observed for the same difference in AM4060 vs. P60 and AM40MML vs. PMML. Arousal ratings only slightly differed between AM4060 and P60. P60 clearly displays the worst tolerability with high arousal and low valence confirming our assumption that PTs at the tinnitus frequency may not qualify for sound therapies. Taken together, these results imply a better tolerability of the AM sounds compared to their PT pendants.

	Value	SE	t-value	p-value
Intercept	90.216	3.485	25.885	0.000
AM1060 - P60 : position	-0.871	0.563	-1.547	0.122
AM4060 - P60 : position	**2.297**	**0.656**	**3.502**	**<0.001**
AM10MML - PMML : position	-0.718	0.653	-1.100	0.271
AM40MML - PMML : position	0.659	0.732	0.900	0.368
AM1060 - P60 : time : position	0.004	0.005	0.812	0.417
AM4060 - P60 : time : position	**-0.018**	**0.006**	**-3.054**	**0.002**
AM10MML - PMML : time : position	0.004	0.006	0.712	0.476
AM40MML - PMML : time : position	-0.007	0.007	-1.069	0.285

Table 2.18: **Results of the linear mixed effects model for the interaction with position**. Degrees of freedom = 1365. Notably, there are significant interactions between condition*position and condition*time*position for the contrast between AM4060 and P60 (in bold). This ancillary analysis elucidates the effect of position in the main model and especially its confounding effect on the contrast between AM4060 and P60 as seen in non-significant differences between these conditions in the main model (see table 2.17).

	Mean	CI Lower	CI Upper	t-value	p-value	p-value (bonf)
Val_AM1060 - Val_P60	1.241	0.511	1.972	3.480	0.002	0.013
Arou_AM1060 - Arou_P60	-0.759	-1.503	-0.014	-2.087	0.046	0.369
Val_AM10MML - Val_PMML	0.552	-0.320	1.424	1.296	0.206	0.999
Arou_AM10MML - Arou_PMML	-0.138	-1.005	0.729	-0.326	0.747	0.999
Val_AM4060 - Val_P60	1.069	0.213	1.925	2.557	0.016	0.130
Arou_AM4060 - Arou_P60	-0.828	-1.502	-0.153	-2.512	0.018	0.144
Val_AM40MML - Val_PMML	1.310	0.384	2.237	2.896	0.007	0.058
Arou_AM40MML - Arou_PMML	-0.724	-1.693	0.245	-1.530	0.137	0.999

Table 2.19: **Paired differences of valence and arousal between stimuli contrasts of interest**. Valence of AM1060 is significantly higher than P60 (t = 3.480, p (bonf) = 0.013 whereas uncorrected results are reported for higher valence of AM4060 vs. P60 (t = 2.557, p = 0.016), higher arousal of P60 vs. AM4060 (t = -2.512, p = 0.018), and higher valence of AM40MML vs. PMML (t = 2.896, p = 0.007). CI = Confidence interval at 95%. Val = Valence. Arou = Arousal.

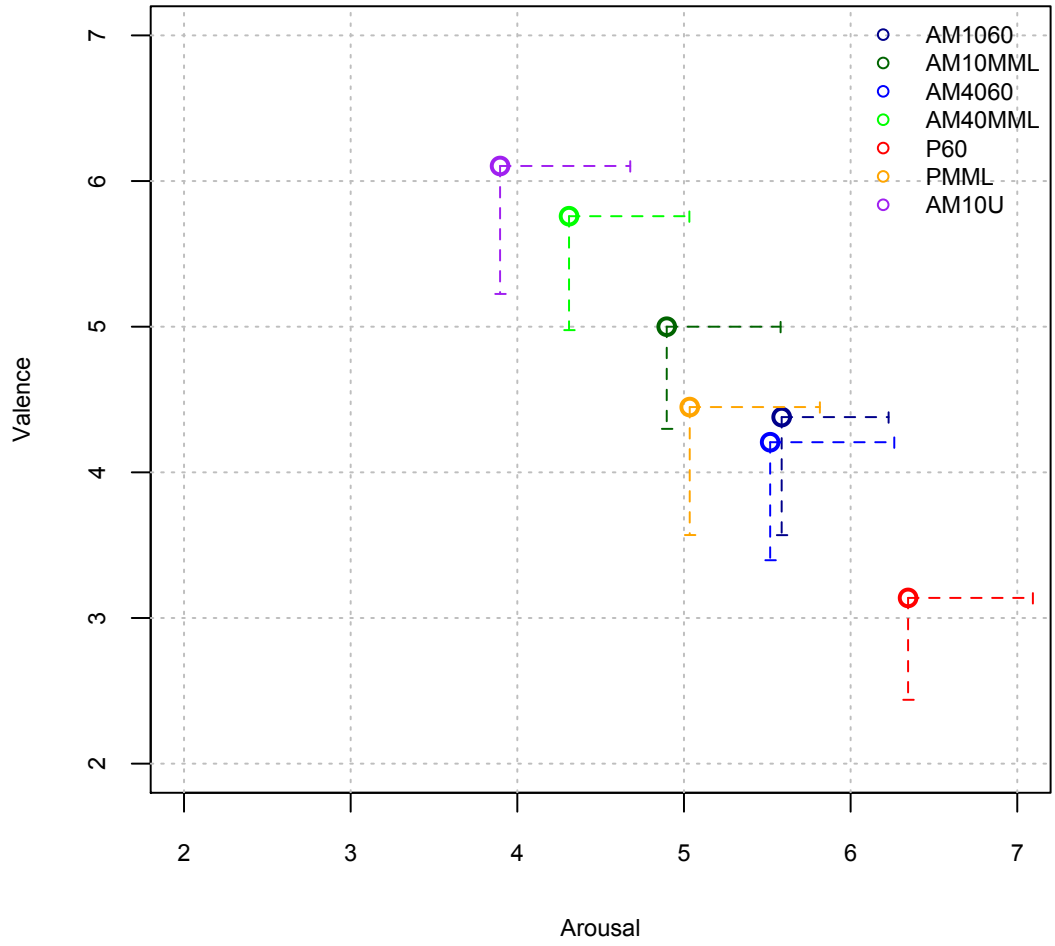

Figure 2.10: **Valence and arousal rating for all stimuli**. Brackets indicating 95% confidence interval for valence and arousal for each condition, respectively. P60 clearly exhibits lowest tolerability mirrored by high arousal and low valence ratings. Significant differences in the contrasts of interest can be observed between valence of AM1060 vs. P60 (t = 3.480, p (bonf) = 0.013), valence AM4060 vs. P60 (t = 2.577, p = 0.130, trend), arousal P60 vs. AM4060 (t = -2.512, p = 0.144, trend), and valence AM40MML vs. PMML (t = 2.896, p = 0.058, trend).

Discussion

This experimental study examined the difference between AM and PT sounds at the tinnitus frequency regarding temporary tinnitus suppression. Specifically, we investigated whether AM sounds with modulation rates of 10 and 40 Hz (5 sounds) induce stronger tinnitus suppression after stimulation than unmodulated PTs (2 sounds) within two stimulation level regimes, namely 60 dB SL and 6 dB above MML (both at the tinnitus frequency). In an additional exploratory analysis we compared both valence and arousal of the different stimuli again comparing AM with unmodulated sounds within the two stimulation level regimes. The aim of these analyses was to further evaluate if AM sounds are suitable to induce tinnitus suppression or residual inhibition and may qualify as a tinnitus sound therapy in the long run.

As hypothesized, the results of the main model taking into account the effect of position (i.e. presentation order of the stimuli) show that 10 Hz AM sounds in the matched tinnitus frequency produce stronger tinnitus suppression after stimulation than unmodulated PTs in the same frequency at stimulation level 60 dB above SL. Looking at different modulation rates (i.e. 40 Hz) and stimulation levels (i.e. 6 dB above MML) we can only report trends in the same direction. In the case of the 40 Hz AM sound at 60 dB SL this may be explained by an (unfortunate) order effect (see tables 2.17 and 2.18). The weak findings of the same contrasts at the lowered stimulation level 6 dB above MML may be explained by the inherent increased sound energy in the 60 dB SL stimuli. Yet, given the observed statistical trends and the considerably large array of similar sound stimuli (i.e., identical regarding their carrier frequency at the matched tinnitus frequency) these results may not come as a surprise but rather could be better elucidated in a sleeker experimental design where presentation level regimes are not mixed within one experiment or experimental block. Furthermore, as no cumulative effect over time (i.e. position) has been found for all stimuli contrary to our reservations, we cautiously interpret the observed order effect as a possible learning effect by the participants (i.e. to attribute better suppression to certain stimuli after identifying their stimuli class membership to either AM or unmodulated sounds). We find this interpretation further plausible as the narrow spectrum of different carrier sounds in the study at hand in contrast to the wide array of carrier sounds in our former study may have introduced the possibility of such learning effects (Neff et al., 2017). In conclusion, we (again) observed better tinnitus suppression proper-

ties for AM sounds (here: modulated PTs at the matched tinnitus frequency) compared to unmodulated pendants.

As for the mechanism of action, neural (or cortical) entrainment may normalize aberrant neural oscillations acting as a correlate of pathologies (e.g., in pain with alpha entrainment (Ecsy et al., 2017) or in schizophrenia with gamma entrainment (Voicikas et al., 2016)). These studies re-evaluate the entrainment hypothesis and clearly evolved away from the use of binaural beats (Oster, 1973). Previous studies showed that sinusoidally AM sounds indeed lead to more pronounced entrainment of cortical neural oscillations (Schwarz and Taylor, 2005; Becher et al., 2014; Draganova et al., 2008) compared to binaural beats naturally limited in modulation depth, modulation rates and carrier frequencies. These findings substantiate the efficacy of entraining neural oscillations and possible subsequent normalization of aberrant activity may be relevant for respective frequency bands in tinnitus (vs. controls) like alpha (Weisz et al., 2005) or gamma (Ashton et al., 2007; Weisz et al., 2007; Sedley et al., 2012). Yet, it has to be determined whether entrainment acts directly on the aberrant oscillatory patterns or rather the observed effect of tinnitus suppression is indirectly measurable as a consequence of the acoustic stimulation with electrophysiology as shown in a recent study by Adamchic and colleagues (Adamchic et al., 2017). Furthermore, the exact role of these frequency bands in the tinnitus pathology, especially alpha and gamma, is still under debate. This is reflected by a reported failed replication of auditory alpha deficiency in tinnitus patients (Zobay et al., 2015) and a proposed inhibitory mechanism of action of gamma oscillations (Sedley et al., 2012, 2016) compared to its proposed role as a correlate of tinnitus presence or loudness (van der Loo et al., 2009; Vanneste et al., 2011). Looking at our findings, we can therefore only guess how the putative gamma entrainment modulated tinnitus perception and abstain from any in-depth modelling at this point. However, we agree with the considerations of Reavis and colleagues (Reavis et al., 2012) that modulated sounds, in contrary to noise or PTs that mostly produce onset and offset auditory cortical activity, may produce "sustained acoustically driven activity that may help restructure cortical firing patterns away from those that generate tinnitus". A comparable model has been postulated where prolonged tinnitus suppression or RI may be explained by inhibition of central synchrony via feedforward projections (Roberts et al., 2010). Regarding possible alpha entrainment, we can not rule out effects of general relaxation (Hartmann et al., 2013)

or mere attentional processes as the alpha band is at the lower bound of the spectrum of entrainable oscillations (Picton et al., 2003; Joris et al., 2004). Nonetheless, analogously to the considerations about the gamma frequency, a similar mechanism of action could be postulated for the alpha frequency as acoustic stimuli with modulation rates in the same frequency may restore normal cortical fire patterns. AM sounds in the alpha band may also have an influence on tinnitus maintenance or attentional networks through a temporary up-modulation of alpha networks driven by the auditory stimulus re-instantiating the shifted brain network homeostasis in tinnitus (decay of wide-spread alpha networks, (related) increase of gamma networks (Schlee et al., 2009a)). At this point, we also embrace the possibility of similar effects produced by stimuli with other modulation rates than 10 or 40 Hz especially covering high frequency bands (e.g., 20-100 Hz (Zeng et al., 2011)). Taking an all-embracing point of view given the various systems of the auditory hierarchy from the inner ear to the brain influenced by acoustic stimulation, it may be conceivable that the observed suppression effect of AM or generally modulated sounds is a conglomerate of altered activity in the auditory pathway, central auditory cortex and widespread cortical network activation as sketched above. To continue this line of research, these entrainment effects should be studied using electro- or magnetoencephalographic methods where direct causal relationships between cortical entrainment and tinnitus suppression can be tested. Beyond that, the influences of the putative entrainment mechanism and the mere RI effect of the carrier sound (here: matched tinnitus frequency) have to be differentiated to better understand the individual and joint mechanisms of action on tinnitus suppression. Finally, effects of lateral inhibition (Mühlnickel et al., 1998; Gerken, 1996) can be ruled out given that the tinnitus frequency was matched reliably and served as the PT carrier sound for all stimuli.

The comparison between arousal and valence ratings between modulated and unmodulated stimuli are similar to the findings in tinnitus suppression as the 10 Hz AM sound at 60 dB SL elicits significantly higher valence but not lower arousal for the AM sound (see table 2.19). Again different modulation rates and stimulation levels only produced trends in differences of arousal and valence between conditions of interest, namely higher valence for AM4060 compared to P60, lower arousal for AM4060 compared to P60 and higher valence for AM40MML compared to PMML (marginally not significant with p = 0.058). Taken together, these results indicate that tolerability for AM sounds seems

to be better compared to PTs, especially in the ratings of valence. On the other hand, it cannot be disputed that the effect is not consistent across the different stimulation levels and modulation rates and almost totally absent in the case of arousal. The latter observation may be further explained by the assumption that arousal is a concept not directly accessible to one's conscious evaluation complicating the abstract task of judging a sound along this particular categorization system. Future studies should consider these shortcomings by elaborating on subjective evaluation of stimuli while still we conclude that the stimuli class of AM sounds was well tolerated by participants, at least for the duration of continuous stimulation (3 minutes).

Despite the mentioned limitations and yet to be better elucidated mechanisms of action, we conclude that, based on our results, AM sounds in the matched tinnitus frequency are highly effective in suppressing tinnitus. This conclusion is substantiated by both better tinnitus suppression or RI effects of AM sounds (especially 10 Hz) and overall better tolerability of this stimulus class by tinnitus sufferers. Future work should focus on understanding the neurophysiological correlates of the observed suppression effects during and after the acoustic stimulation as well as on testing long-term effects of the approach. Given the the efficacy, tolerability and simplicity of use we propose the studied stimulus class as a suitable candidate for long-term tinnitus sound therapy.

Acknowledgements

This research was supported by by the University Research Priority Program 'Dynamics of Healthy Aging' of the University of Zurich, the 'Fonds zur Förderung des akademischen Nachwuchses' (FAN) des 'Zürcher Universitätsvereins' (ZUNIV) and by TINNET - COST Action BM1306 'Better Understanding the Heterogeneity of Tinnitus to Improve and Develop New Treatments'.

Author contributions statement

P.N. conceived the experiment, analysed the results and wrote the manuscript. L.Z. conducted the experiment. M.M., B.L. and M.S. conceived the experiment. W.S. conceived the experiment and analysed the results. All authors reviewed the manuscript.

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3 Discussion

In the final chapter of this thesis, key findings of the empirical studies will be shortly discussed and integrated into the concurrent state of research. Finally, an outlook into ongoing and (needed) future research is given.

3.1 Differential Tinnitus-related Neuroplastic/-static Alterations of Cortical Thickness and Surface Area

Neuroanatomical studies in tinnitus have suffered from various shortcomings resulting in an unsatisfactory and inconsistent picture of structural brain imprints of tinnitus in the brain. Recent technological and methodological advancements allowed for more in-depth analysis and if performed carefully following respective standards (Adjamian et al., 2014; Ridgway et al., 2008) as well as a philosophy of transparent reporting, may in consequence allow for a well-replicated and consistent big picture of macroscopic neuroanatomy related to tinnitus. To both perform along these advancements and established recommendations, study 1 (Meyer, Neff, et al., 2016) re-analyzed a large sample of TI (Schecklmann et al., 2013) with a SBM method, namely the observer-independent fully automated cortical surface reconstruction pipeline of FreeSurfer (Fischl et al., 1999a,b; Fischl and Dale, 2000; Fischl et al., 2001, 2002), to both replicate and extend previous findings.

Open questions for study 1: *Are there alterations in auditory cortex related to tinnitus which can be detected using SBM and correlational analyses within a large sample of TI? Do these putative alterations exert differential patterns of CT, CSA, or CV and can these patterns extend former findings of reduced cortical gray matter in auditory cortex? Is there a relationship between key tinnitus parameters like distress or duration and cortical morphology? Do these relationships inform us about neuroplasticity or predisposition of tinnitus with alterations in CT or CSA, respectively?*

To address these questions, we hypothesized that we would replicate the findings of de-

creased cortical GM (i.e., in CV) related to tinnitus distress of the former study (Schecklmann et al., 2013). The results provided support for this hypothesis with negative correlations between CV and tinnitus distress in bilateral auditory cortex (two clusters) even with comparable effect sizes (r = .25 to .29) in regard to the former study. Beyond that, we could identify CSA as the driving factor behind this reduction in auditory fields extending former results and confirming considerations about reduced GM in auditory cortex being a possible indicator of general (Schneider et al., 2009) or distress-related vulnerability to tinnitus. With the vast majority of included TI exhibiting bilateral tinnitus, our findings of CSA (possibly related to early development or even genetic predisposition (Storsve et al., 2014; Winkler et al., 2010)) reductions could be cautiously interpreted as being related to the observation of genetic heritability of bilateral tinnitus (Maas et al., 2017). Yet, with the field of genetics just recently entering tinnitus research and yet to be solidly replicated findings of smaller CSA in auditory fields, it is definitely to early to postulate concrete relationships between the two methodologies or implications for e.g. diagnostic procedures. Moreover, it has to be stretched, also looking at similar alterations of CSA in non-auditory regions, that the findings for CSA controlled for all other possible influences on this cortical measure are convincing, despite weak effect sizes. While the pattern of negative correlations between distress and CSA might be impressive and present at various hubs within the tinnitus network of the brain, we still observed a single positive correlation of distress with CT in cingulate cortex structures, while CSA was reduced in the same region. The finding of increased CT may therefore be reflecting actual neuroplastic changes of distress instead of the omnipresent CSA reductions possibly indicative of predispositional factors. Still, it can not be ruled out that these bi-directional alterations of the putatively independent neuroanatomical traits of CT and CSA are an artifact, which is further supported by the weak effect sizes in this subanalysis. Looking at the differential pattern of correlations between tinnitus duration and exclusively CT, the impression that tinnitus distress is related to predispositions in cortical architecture solidified. Furthermore, with a decrease in CT in sgACC and a concomitant increase in left temporal secondary auditory fields possibly related to sound (here: tinnitus) awareness (De Ridder et al., 2014), the theorized deficient noise canceling system in the frontostriatal gating model (Rauschecker et al., 2015) may have received a convincing neuroplastic correlates while we could not replicate or confirm any emotional involvement (e.g., (Leaver et al.,

2012)). According to our data, the deficient noise canceling system therefore emerges with time or ongoing tinnitus chronification, an observation which is difficult to both reconcile with the frontostriatal gating model or other data at this time.

Future research is certainly needed here to understand the complex interplay of tinnitus generation, chronification, structural brain imprints, genetics, plasticity, and various tinnitus parameters. Certainly, SBM should play a key role here and large, ideally pooled, samples should be carefully analyzed following established standards in the tinnitus research initiatives.

3.2 Exploratory Study on Feasibility of a Novel Acoustic Stimulation Paradigm for Tinnitus Suppression Using Amplitude Modulated Sounds

Acoustic stimulations for tinnitus relief have a long tradition and are still, in combination with counseling, viable ways of therapeutic intervention in tinnitus (Baguley, 2003; Henry et al., 2005a,b). Up until recently, only continuous sounds like broad-band noise, NBN, and PT have been used, with the exception of two prominent approaches using either a notch filter on music (Pantev et al., 2012) or short tones randomly presented around the tinnitus frequency (Tass et al., 2012). Only recently, a single study aimed at probing AM and FM sounds modulated with 40 Hz for tinnitus suppression or RI in four frequency bins. To further probe the field of modulated sounds, study 2 (Neff et al., 2017) aimed at exploring various carrier sounds and frequencies with 10 Hz AM sounds. A sum total of seven stimuli in a first and two manipulated stimuli in a second block were presented to TI, while tinnitus suppression relative to the initial tinnitus loudness was rated after stimulation offset. Beyond efficacy of this novel stimulus class, also safety and tolerability were also investigated.

Open questions for study 2: *Are amplitude modulated (10 Hz) sound exerting better short-term tinnitus suppression than their unmodulated pendants? Is this acoustic stimulation approach generally feasible and safe?*

In addressing the first question, it has to be stated that within the exploratory study at hand and the contrasts of main interest, only the comparison between the 10 Hz AM sound matched and the pink noise condition produced a significant result. Notably, a direct

comparison between the two stimuli is not possible as pink noise certainly possesses less spectral energy in frequency regions around the tinnitus compared to the well-matched PT. On the other hand, the broader frequency distribution of pink noise in contrast to the tight distribution of PT frequency spectra may partly enable lateral inhibition reducing the tinnitus percept. Furthermore hearing loss may further limit the efficacy of the noise sound, especially in the upper parts of its frequency distribution. Therefore, future studies or iterations should consider the use of stimuli matched for loudness over time (e.g., noise and PT sounds made comparable with respect to their root mean square (RMS) level over a certain time period) and ideally also adjusted for hearing loss (amplification of respective frequencies). Looking at the contrast between modulated and unmodulated PT in the matched frequency, only a trend of the modulated sound exerting larger tinnitus suppression can be reported. Interestingly, the stimulus of combined AM and FM, in which every AM cycle contains a FM sweep from 1 Hz up to the tinnitus frequency, resulting in ten sweeps per second with the sweep being the loudest in the tinnitus frequency (thus coinciding with the maximal amplitude of AM), performs equally good as the matched AM PT compared to pink noise. This fact possibly also led the participants to favor the AMFM stimulus over all others for block 2. Manipulations of the matched AM PT stimulus in stimulation level and length partly confirmed our hypotheses that these parameters do alter tinnitus suppression in the expected direction, but effects were rather weak in the case of stimulation level manipulations.

In conclusion, results could show that AM sounds at a 10 Hz modulation rate do exert slightly better or similar tinnitus suppression than unmodulated sounds. Given the inclusion of the whole study sample and no transformation of the data compared to the strategy of Reavis et al. (2012), results indicate solid tinnitus suppression of the novel stimuli class. Yet, the stimulation level regime is not comparable to that of the former study with stimuli presented 60 dB SL, thus producing larger tinnitus suppression or RI as demonstrated by complete abolishment of tinnitus in a third of the participants after stimulation offset at stimulation levels of 65 dB SL up to 95 dB SPL (Roberts et al., 2006).

Regarding the second question, it can be confidently stated that the study has proven that the approach is both feasible and safe. As a matter of fact, we encountered only a single early study termination initiated by a participant due to basic motivational issues and not issues related to the acoustic stimulation. Furthermore, with a few exceptions,

tinnitus loudness never increased after or during the stimulation and, in case of an increase, diminished shortly after. TI behind these exceptions may be suffering from abnormal loudness recruitment or hyperacusis and, therefore, have not 'properly' responded to stimulation (Reavis et al., 2012). Finally, contrast between tinnitus questionnaires before and after the entirety of the experimental produced significant reductions in subjective loudness, and tinnitus distress. While these results should not be over-interpreted given various methodological shortcomings (e.g., no discrimination between modulated and unmodulated sounds), they certainly point at the safety and feasibility of the approach. Future studies should therefore continue along these lines and probably increase stimulation length. Besides all these considerations, it remains difficult at this point to compare the results or generally the approach to other work given the inexistence of respective literature and the novelty of the approach. Notably, with all the similarities this study may have in common with the study of Reavis et al. (2012), differences like stimulation level, stimulation rates, and frequency bins instead of matching immensely limit the comparison on every parametric dimension. Further, speculations about entrainment effects (e.g., in pain (Ecsy et al., 2017)) or normalization of aberrant central activity (Reavis et al., 2012) have to be studied using MEEG methods.

3.3 Comparison of Matched Tinnitus Pure Tones at Different Modulation Rates and Stimulation Level Regimes

To follow up on study 2, study 3 (Neff et al., submitted) has synthesized insights from the former exploratory study and theoretical considerations. First, the influence of different modulation rates on tinnitus suppression was tested, namely 10 Hz like as in the former study and 40 Hz (Reavis et al., 2012). Second, stimuli were presented in two stimulation level regimes with half of the stimuli presented at 60 dB SL comparable to (Neff et al., 2017; Roberts et al., 2006) and the other half above the minimal masking level slightly higher than in Reavis et al. (2012). Taking up the stimulus from the former study exerting the most tinnitus suppression, the carrier frequency for all of the stimuli was the matched tinnitus frequency and the carrier sound a PT. An entirety of seven stimuli was then finally tested in a single block and the different stimuli were compared with valence and arousal ratings using manikins (Bradley and Lang, 1994).

Open questions for study 3: *Do amplitude modulated sounds with different modulation rates exert differential short-term tinnitus suppression than their unmodulated pendants? Is there a difference in tinnitus suppression when the stimulation level is manipulated? How is the tolerability for the different stimuli elicited by valence and arousal ratings?*

In addressing the first two questions, results of study 3 indicate that both 10 Hz and 40 Hz produced better short-term tinnitus suppression than their unmodulated PT pendants. There was a tendency that 10 Hz showed slightly better suppression than 40 Hz as visible in the final results of the main model corrected for multiple comparisons, in which only the 10 Hz AM PT at 60 dB SL could significantly outperform its unmodulated pendant. Of special note, unlike in study 2, we encountered a position effect (i.e., presentation order of the seven stimuli within the experiment) in the data which in consequence led to a change in the linear mixed effects statistical model as position was added as a covariate. This circumstance was rather sobering given the otherwise almost too amazing results which were perfectly aligned to all hypotheses and assumptions. Yet, the pattern of these results of the preliminary model, exhibiting significantly better suppression for all of the AM stimuli with a slight advantage for 10 Hz, also in both stimulation level regimes with an advantage for the 60 dB SL stimuli and again 10 Hz, is still visible in the final results of the optimally fitted model with position as a covariate while lacking statistical power. Consolingly, still, the results of this study tie in elegantly and convincingly to the former small body of research (Reavis et al., 2012; Neff et al., 2017) in primarily boiling down the overarching question if and how AM stimuli may be superior to unmodulated sounds into the most convincing data to date. Unlike other endeavors in tinnitus research, for example the probably already 'casketed' branch of macroscopic neuroanatomy, the young field of modulated sounds for tinnitus suppression and potential future sound therapy seems to develop in a consistent and hypothesis-driven manner. This development can be certainly regarded as constructive 'slow science' towards a solid end goal compared to hyped quick shots disappearing from the stage of acoustic stimulation ever so hastily as they have appeared in the first place.

Looking at the final question concerning tolerability and again the (related) issues of feasibility and safety, the resulting data is indicative of better tolerability and thus probably acceptance for the AM sounds, especially 10 Hz, compared to the PT pendants. This

finding is again in line with our considerations and concrete expectations, contributing further data to the assumption that this novel stimuli class may be suitable for therapeutic sound therapy for tinnitus relief. Concretely, particularly valence ratings for the 10 Hz AM stimuli compared to the PT pendant showed respective statistical power. Given that our studies where the first to assess these kinds of reactionary subjective ratings in this stimuli class, it is impossible, at this point, to discuss these results in any other context than our own data. Moreover, with the stimuli being deliberately reduced to their core parameters of interest with no effort spent to design them aesthetically appealing and with the relatively short stimulation periods, it is deemed too early to interpret these findings in any more depth at this time. Future studies should therefore certainly test these subjective ratings with longer stimulation periods and further develop the stimuli class towards attractive sounds for daily, individual use.

3.4 Conclusion and Outlook

The thesis at hand including the presented studies is situated in a complex research area tackling a chronic phantom symptom with an immense variety of manifestations, causes, and mechanisms. After almost a hundred years of modern science research (Wegel, 1931; Jastreboff, 1990) and scientific interest even dating back to ancient or antique times (Dietrich, 2004), the haunting and tantalizing chronic phantom sound is still elusive. Unfortunately for tinnitus sufferers, all waiting for relief, even modern science and technological advancements can not provide them with anything more than some (probably often unspecific (Baguley, 2003)) relief, often with no guarantee. This unfortunate situation calls for intensified efforts deploying everything from up to date methodologies, multidisciplinarity, international collaborations and data pooling, and, last but not least, all the best intentions of integer and committed scientists.

Looking at the studies within this thesis, conducted along the lines of the above suggestions, it is concluded that all of them, to the best of the author's current abilities, can be regarded as perforce constructive advancements of research in the tinnitus field. While there might be no direct link between basic research neuroanatomy and the development of a novel acoustic stimuli class aiming at resetting tinnitus-related neurophysiological activity, certainly fruitful interactions can be identified. First of all, as Langguth et al. (2012) noted, neuromodulation (here: expanded to putative neuronal mechanism of the

AM stimuli class) and neuroimaging are fruitful methods, both used independently and jointly, to probe the inherent heterogeneity of tinnitus and to better understand its causes. This can be thought ahead to the point, where neuromodulatory techniques can actually test causalities, instead of only relying on correlations, in adequate research frameworks. Second, concrete combined use of neuroanatomical (but also neurophysiological) methods and auditory stimulation can be envisioned to probe long-term effects and neuroplastic effects of the novel acoustic stimuli class. Given the already obtained observations of differential brain imprints in auditory and non-auditory regions of various differential parameters of CT, CSA, and CV (Adjamian et al., 2014; Meyer et al., 2016; Yoo et al., 2016; Allan et al., 2016) such research projects might indeed lead to a better understanding of tinnitus in the spirit of Langguth et al. (2012).

Recently, the SBM method sported by FreeSurfer took a giant step towards even more precise and accurate cortical surface reconstruction by extending their analysis pipeline with machine (deep) learning (Wachinger et al., 2017). For tinnitus research, this might be more than good news as this new approach possibly helps to overcome the heterogeneous findings and general weak effect sizes (Adjamian et al., 2014; Schecklmann et al., 2013; Vanneste et al., 2015; Yoo et al., 2016; Allan et al., 2016; Meyer et al., 2016). On the other hand, international data pooling for a large scale collaborative study of neuroanatomy, possibly also applying observer-independent machine learning algorithms like classifiers, is in planning within the TINNET research initiative (http://tinnet.tinnitusresearch.net/). Furthermore, longitudinal studies in neuroanatomy, but also with other neuroscientific methods (with the exception of test-retest study of MEEG data (Pierzycki et al., 2016)), are largely absent and deemed as critical to understand (early) chronification mechanisms of tinnitus especially. Beyond that, longitudinal studies could further the understanding of the role of (healthy) aging in the context of tinnitus as up until now only cross-sectional studies looked at this issue (e.g., (Yoo et al., 2016)).

Regarding the future development of the introduced novel stimuli class of AM sounds, or more generally put modulated or patterned sounds, it is regarded as mandatory, besides the longer stimulation periods and testing of the approach with MEEG methods, to consider the creation of aesthetically more appealing (e.g., more musical) sounds or embed the approach as modulations of existing sounds (Pantev et al., 2012). One feasible avenue could be the use of generative music (Nierhaus, 2009) which could be custom-tailored to

both fit the needs of aesthetical appeal and optimal modulation of relevant frequencies. The author's musical background and loose affiliation to an academic computer music institute could certainly ease such endeavors. Coming back to ongoing basic research, the approach of AM stimuli for tinnitus relief has in the meantime be further iterated and data collection of a study in more than 30 participants with both tonal and noise-like tinnitus has been successfully completed. Furthermore, to establish practical methods of assessing neural correlates (MEEG) of the acoustic stimulation approach, much needed after successful behavioral 'proof of principle' studies presented in this thesis, forces were joined under the umbrella of TINNET and a trilateral short-term scientific mission is on its way (http://tinnet.tinnitusresearch.net/images/pdf/STSM/STSM_ZH_astim_EEG.pdf). Following the establishment of these methods and workflows, the approach can be tested at various centers in parallel to increase the availability of data as similarly done in the RI study of Roberts et al. (2008).

To close, all the collaboration and data pooling efforts sketched above should be concretized to finally usher in a new aera of big data science in tinnitus, which is much needed given the heterogeneous and inconsistent findings. Database projects like the newly established international tinnitus database (https://www.tinnitus-database.de/welcome) should be used for these purposes. All in all, while still being haunted by many issues, future research in tinnitus might be more productive and insightful when continuing along these inspiringly fresh avenues of well-informed collaborative efforts.

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5 Curriculum Vitae

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Peer-reviewed publications

Meyer, M., Luethi, M. S., **Neff, P.**, Langer, N., & Büchi, S. (**2014**). Disentangling Tinnitus Distress and Tinnitus Presence by Means of EEG Power Analysis. Neural plasticity, 2014.
Meyer, M.*, **Neff, P.***, Liem, F., Kleinjung, T., Weidt, S., Langguth, B., & Schecklmann, M. (**2016**). Differential tinnitus-related neuroplastic alterations of cortical thickness and surface area. Hearing Research, 342, 1-12. * Equal contribution
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Neff, P., Michels, J., Meyer, M., Schecklmann, M., Langguth, B., & Schlee, W. (**2017**). 10 Hz amplitude modulated sounds induce short-term tinnitus suppression. Frontiers in Aging Neuroscience. doi: 10.3389/fnagi.2017.00130